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TABLE OF CONTENTS

	Author	Page
Population genetic studies on sodium and potassium in sugar beets (*Beta vulgaris* L.)	*John W. Dudley* *LeRoy Powers*	97
Incidence of Rhizoctonia crown rot of sugar beets in irrigated crop rotation	*M. L. Schuster* *L. Harris*	128
Determination of microorganisms in sugar products by the Millipore method	*H. E. Halden* *D. D. Leethem* *F. G. Eis*	137
Bulk and liquid sugar production at the Brookfield (Chicago) Terminal	*L. T. Jenesn* *Whitney Newton II*	143
An investigation of the effects of various methods of herbicide application on weed control in sugar beets	*J. D. Bandeen* *G. E. Jones* *C. M. Switzer*	151
Selection of sugar beets for tolerance to endothal herbicide	*R. T. Nelson* *R. R. Wood* *R. K. Oldemeyer*	155
Further studies on the control of weeds in sugar beets with herbicides	*J. D. Bandeen* *G. E. Jones* *C. M. Switzer*	160
Some effects of virus yellows on sugar beet processing quality	*A. E. Goodban* *A. I. Morgan* *R. Teranishi* *H. G. Walker, Jr.* *R. M. McCready*	164
The design, operation and economic evaluation of a gas-fired lime kiln	*Sylvester M. Heiner*	168
Evaluation of combining ability in self-fertile lines of sugar beets using male-sterile testers	*Donald L. Oldemeyer* *George E. Rush*	175
Notes Section		186

Population Genetic Studies on Sodium and Potassium In Sugar Beets (Beta Vulgaris L.)[1]

JOHN W. DUDLEY AND LEROY POWERS[2, 3]

Received for publication November 25, 1959

Introduction

With the recent increase in the use of nitrogen fertilizer in sugar beet production and the resultant decrease in processing quality, interest has been aroused in the possibility of altering the chemical composition of the sugar beet root by breeding methods. To obtain information on this problem population genetic studies at different levels of soil fertility were conducted. The results of studies carried out in 1956 for percentage sucrose, weight of root, and No_3-N in the petioles were reported by Powers, Robertson, Whitney, and Schmehl (13)[4]. The results of the study of total nitrogen in the roots from this same experiment were reported by Payne, Powers, and Maag (4). The purpose of this paper is to report the results of the analysis of data obtained for the sodium and potassium contents of these same roots. The objective of the study was to obtain information on the genetic and breeding behavior of sodium and potassium in sugar beet roots grown at different soil-fertility levels.

Materials and Methods

The experimental design used in this study is a split-split plot with 40 replications. Fertilizer treatments are the main plots, populations the sub plots, and locations within the single row of each population the sub-sub plots. The fertilizer treatments, populations, and locations are described in detail by Powers, et al. (13).

The fertilizer treatments are fertilization and non-fertilization. The fertilized plots received 100 pounds of N and 250 pounds of P_2O_5 per acre on April 4, 1956, and an additional 100 pounds of N per acre on June 26.

The experimental plant material consists of six populations: A54-1; A54-1, BB; 50-406, BB; 50-406; F_1 hybrid; and 52-307. A54-1 is a commercial variety. A54-1, BB is a broad-base derivative of A54-1 produced by exposing 25 mother beets of

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[1] Cooperative investigations of the Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, the Colorado Agricultural Experiment Station, the Beet Sugar Development Foundation, and the Holly Sugar Corporation. Approved by the Colorado Agricultural Experiment Station for publication as Scientific Series Article No. 589.

[2] Geneticists, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture.

[3] The authors wish to acknowledge the contribution of the data used in this study by the Research Department of the Holly Sugar Corporation.

[4] Numbers in parentheses refer to literature cited.

A54-1 to pollen from 22 other populations. Population 50-406, BB is a broad-base derivative of 50-406 produced in a similar manner. Populations 50-406 and 52-307 are inbred lines, while the F_1 hybrid is the cross 50-406 x 52-307. Populations A54-1, BB; A54-1; and 50-406, BB are heterogeneous and heterozygous and will be referred to as the heterogeneous populations. Populations 50-406, F_1 hybrid, and 52-307 will be referred to as the homogeneous populations. Comparisons between the heterogeneous and the homogeneous populations will be called comparisons between geneities.

Each location within a row consisted of a single plant. Since the rows were planted from north to south, the differences between locations represent any north-south gradient in the field.

Each plot was bordered by a row of A54-1. The rows were spaced 22 inches apart and the plants were thinned to a spacing of 20 inches within the row. After thinning there were 12 plants in each plot. Data were taken on eight plants per plot with the end plants being discarded at the time of harvest.

Sodium and potassium concentrations of the beet juices were determined with the Beckman Model DU Spectrophotometer and the flame attachment. Lead acetate filtrate samples were analyzed without dilution or addition of an internal standard. Sodium and potassium concentrations were determined from the transmission at 589 and 768 μ respectively, with the numerical values in ppm being taken from a standard curve. These values were then converted to percent on beets by the appropriate calculations.

The standard curves were prepared by plotting the transmission on varying known amounts of the element in question. In addition to the sodium and potassium, other known impurities were incorporated in the standard solutions as follows: Ca, 50 ppm; Mg, 50 ppm; sucrose, 2%; lead acetate solution, 6.25 ml/100 ml; K, 200 ppm (in Na standard); and Na, 50 ppm (in K standard). Periodically throughout the runs the operation of the spectrophotometer was checked by running a reference standard of this composition (including 200 ppm K and 50 ppm Na).

The data were recorded as percent on beets of sodium and potassium. For ease of handling, the data were changed to parts per 100,000. Three observations are missing for each character. The plot mean was used to replace each missing observation and the appropriate degrees of freedom were reduced to compensate for the missing observations. Since the frequency distributions

are not following the normal curve on an arithmetic scale, the data were transformed to logarithms of parts per 100,000. The transformed data were used in the statistical analyses.

Questions which are of interest in this study are whether differences between main effects exist, whether there are interactions between main effects, whether there are differences between mean variances, whether the residual variances are different from zero, and if so, which are greater. If the residual variances are different from zero the frequency distributions are of interest.

The analysis of variance is used to test the differences between main effects and interactions and to test differences between mean variances. Chi-square is used to test differences between frequency distributions. Regression of variances on means is used to estimate the environmental variances for the sodium data. For details of the statistical methods used see Powers (6, 9); Powers, Locke and Garrett (10); Powers, et al. (13); Powers, Robertson, and Clark (11); and Powers, Robertson, and Remmenga (12). Where a difference is said to be significant, the odds are at least 19:1 against the difference being due to chance unless otherwise stated.

Results

The results will be discussed under three main headings; means, variances, and frequency distributions.

Means

Analyses of variance

The mean squares and F values from the analyses of variance are shown in Table 1. Because differences between geneities exist for some of the interactions involving populations, the sum of squares for populations is broken down into geneities, heterogeneous populations, and homogeneous populations. Similarly the interactions involving populations except R x P x T and the remainder are broken down into their component parts. In Table 1, G represents geneities, He represents heterogeneous populations, and Ho represents homogeneous populations. The error mean squares used in making the F tests are designated by the small letters in columns 2, 6, and 7. The presence of significant differences between main effects or interactions can be determined by comparing the F values in columns 6 and 7 with the appropriate tabular F values in columns 8, 9, 10, and 11.

The F values for all main effects except locations exceed the one percent level for both characters. For sodium the F value for locations exceeds the one percent level, but for potassium no significant differences between locations were found.

Table 1.—Analyses of Variance for Logarithms of Parts per 100,000 of Sodium and Potassium.

Source of Variation	Smaller Mean Square	Mean Square		D.F.[1]	F Value[3]		Tabular F			
		Sodium	Potassium		Sodium	Potassium	Sodium		Potassium	
							5%	1%	5%	1%
Replications		4.5596	0.0891	39	89.05f	6.41f	1.45	1.69	1.45	1.69
Populations		3.9578	1.0777	5	59.43a	77.53f	2.26	3.11	2.26	3.11
Geneities		1.1179	2.1100	1	17.25b	151.80f	4.10	7.35	3.89	6.76
Heterogeneous		5.1561	1.1381	2	59.54c	81.88f	3.11	4.88	3.04	4.71
Homogeneous		4.1794	0.5012	2	87.80d	36.06f	3.11	4.88	3.04	4.71
Treatments		74.4505	1.1254	1	179.53e	53.34e	4.10	7.35	4.10	7.35
Locations		0.0792	0.0073	7	2.82g	1.20g	2.01	2.64	2.01	2.64
R x P	a	0.0666	0.0148	195	1.30f	1.06f	1.26	1.39	1.26	1.39
R x G	b	0.0648	0.0177	39	1.27f	1.27f	1.45	1.69	1.45	1.69
R x He	c	0.0866	0.0158	78	1.69f	1.14f	1.35	1.53	1.35	1.53
R x Ho	d	0.0476	0.0125	78	f	f	1.35	1.53	1.35	1.53
R x T	e	0.4147	0.0211	39	8.10f	1.52f	1.45	1.69	1.45	1.69
P x T		0.2365	0.1978	5	4.62f	14.23f	2.26	3.11	2.26	3.11
G x T		0.0099	0.1170	1	f	8.42f	3.89	6.76	3.89	6.76
He x T		0.4626	0.0141	2	9.04f	1.01f	3.04	4.71	3.04	4.71
Ho x T		0.1257	0.4220	2	2.42f	30.36f	3.04	4.71	3.04	4.71
R x P x T	f	0.0512	0.0139	195	1.82g	2.28g	1.17	1.25	1.17	1.25
Remainder	g	0.0281	0.0061	5350[2]						

1. D.F. designates degree of freedom.
2. Three degrees of freedom are removed from the remainder to account for three missing observations.
3. The small letters after the F values indicate the error mean square used in calculating F.

The R x P interaction in the sodium data is significant at the five percent level. When this interaction is broken down into its component parts, the R x He interaction is significant at the one percent level but neither the R x G nor the R x Ho interaction is significant. None of the components of the R x P interaction in the potassium data is significant.

The R x T interaction exceeds the one percent level for sodium and the five percent level for potassium.

The P x T interaction exceeds the one percent level for both sodium and potassium. When this interaction is broken down for sodium, the He x T component is significant at the one percent level, but neither of the other components is significant. For potassium both the G x T and the Ho x T interactions are significant at the one percent level, but the He x T interaction is not significant.

The R x P x T interaction is significant at the 1 percent level for both characters when tested against the pooled interactions with locations.

Main effects

The means for main effects are presented in Table 2. For ease of presentation and to have a basis for comparison with the No_3-N petiole analysis reported by Powers, et al. (13), the replications are grouped into five groups and means are reported as group means. From Table 2 it can be seen that the first two replication groups have approximately the same mean sodium

Table 2.—Means of Logarithms of Parts per 100,000 of Sodium and Potassium for Replication Groups, Populations, and Treatments.

Main Effects	Sodium	Potassium
Replication groups		
1-8	1.1721	2.0308
9-16	1.1879	2.0213
17-24	1.2995	2.0088
25-32	1.5206	2.0419
33-40	1.6745	2.0820
Populations		
A54-1	1.4482	2.0940
A54-1, BB	1.4309	2.0741
50-406, BB	1.2848	2.0130
50-406	1.3016	2.0440
F_1 hybrid	1.3130	1.9890
52-307	1.4469	2.0074
Treatments		
Fertilized	1.5102	2.0541
Non-fertilized	1.2317	2.0198

content, the third group is slightly but significantly higher while increases in the last two groups are sharp, indicating a gradient of increasing sodium content from north to south in the field. There is a small, significant decrease in potassium content from replication group 1-8 to group 17-24 and a marked increase in the last two groups.

Examination of the population means reveals dominance for low sodium with 50-406 and the F_1 hybrid both significantly lower than 52-307. Population 50-406, BB is not significantly different from 50-406. The F_1 hybrid has a significantly lower potassium content than either 50-406 or 52-307 indicating heterosis for low potassium. Population 50-406, BB is significantly lower in potassium than 50-406. This is another indication of either dominance or heterosis for low potassium.

The means for the fertilized plots are higher than those for the non-fertilized plots for both sodium and potassium.

First-order interactions

The means for the interaction of replications x treatments are shown in Table 3. The nature of this interaction can be seen for the sodium data by examining the difference between the fertilized and non-fertilized plots for replication groups 1-8 and 33-40. For group 1-8 the difference is 0.3623, while for group 33-40 it is 0.2123. This interaction is of practical significance because it indicates that the magnitude of the response to fertilization, as measured by sodium content, depends on the original fertility level of the soil. That a gradient did exist is shown by Powers, et al. (13).

Table 3.—Means of Logarithms of Parts per 100,000 of Sodium and Potassium for the Interaction of Replication Groups x Treatments.

Character and Treatment	Replication Group				
	1-8	9-16	17-24	25-32	33-40
Sodium					
Non-fertilized	0.9910	1.0328	1.1792	1.3870	1.5683
Fertilized	1.3533	1.3430	1.4198	1.6541	1.7806
Potassium					
Non-fertilized	2.0089	2.0034	1.9926	2.0374	2.0568
Fertilized	2.0527	2.0391	2.0249	2.0464	2.1071

The means for the interaction of replications x populations are shown in Table 4. The interaction of replications with heterogeneous populations is the only significant component of this interaction in the sodium data. This interaction results primarily from the failure of A54-1, BB to increase gradually

Table 4.—Means of Logarithms of Parts per 100,000 of Sodium and Potassium for the Interaction of Replication Groups x Populations.

Character and Replication Group	Population					
	A54-1	A54-1, BB	50-406, BB	50-406	F_1 hybrid	52-307
Sodium						
1-8	1.2692	1.2959	1.0421	1.0823	1.0764	1.2668
9-16	1.2863	1.2108	1.1307	1.1254	1.1037	1.2706
17-24	1.3702	1.3339	1.2090	1.2723	1.2495	1.3622
25-32	1.5607	1.6037	1.4320	1.4549	1.4831	1.5890
33-40	1.7546	1.7104	1.6103	1.5731	1.6525	1.7460
Potassium						
1-8	2.0904	2.0577	1.9923	2.0284	1.9983	2.0177
9-16	2.0517	2.0552	2.0068	2.0324	1.9791	2.0024
17-24	2.0609	2.0367	1.9884	2.0099	1.9653	1.9915
25-32	2.1120	2.0863	1.9967	2.0525	1.9835	2.0204
33-40	2.1548	2.1348	2.0811	2.0969	2.0189	2.0053

in sodium content from the first replication group to the last, whereas the other heterogeneous populations do increase gradually. In the potassium data none of the components of the interaction of replications with populations is significant.

The means for the interaction of populations x treatments are shown in Table 5. The only part of the interaction of populations x treatments that is significant in the sodium data is the interaction of heterogeneous populations with treatments. This interaction is apparently the result of the difference between fertilized and non-fertilized plots being only 0.2257 for 50-406, BB while it is 0.2864 and 0.3329 for A54-1 and A54-1, BB, respectively.

In the potassium data the interaction of geneities x treatments and of homogeneous populations x treatments are both significant at the one percent level. Examination of Table 5

Table 5.—Means of Logarithms of Parts per 100,000 of Sodium and Potassium for the Interaction of Populations x Treatments.

Character and Treatment	Population					
	A54-1	A54-1, BB	50-406, BB	50-406	F_1 hybrid	52-307
Sodium						
Non-fertilized	1.3050	1.2645	1.1720	1.1487	1.1788	1.3212
Fertilized	1.5914	1.5974	1.3977	1.4545	1.4472	1.5727
Potassium						
Non-fertilized	2.0767	2.0485	1.9880	2.0070	1.9770	2.0217
Fertilized	2.1112	2.0998	2.0381	2.0811	2.0011	1.9931

reveals that the interaction of homogeneous populations x treatments is caused by a significant increase in potassium from the non-fertilized to the fertilized plots in population 50-406 and a significant decrease from the non-fertilized to the fertilized plots for 52-307. The interaction of geneities with treatments is a result of the interaction of homogeneous populations with treatments since the increase in potassium from the non-fertilized to the fertilized plots for 50-406 and the F_1 hybrid is partly offset by the decrease of 52-307. The net result is a mean difference of 0.0232 between fertility levels for the homogeneous populations as compared with 0.0453 for the heterogeneous populations.

Second-order interaction

The means for the interaction of replication groups x populations x treatments are shown in Table 6. This interaction is of interest in the sodium data because of the effect on dominance relationships in the F_1 hybrid. For the first four replication groups of both the non-fertilized and fertilized plots the means for 50-406 and the F_1 hybrid are not significantly different while in group 33-40 the F_1 mean is intermediate between those for 50-406 and 52-307. Thus depending upon the replication group which is studied, either no dominance or complete dominance for low sodium is indicated.

The F_1 hybrid is significantly lower in potassium than either parent on replication group 25-32 in the non-fertilized plots. In 3 of the other 4 replication groups on the non-fertilized plots the F_1 hybrid was slightly lower than the low parent but none of these differences was statistically significant. The low parent on the non-fertilized plots is 50-406 for all replication groups except 33-40. In replication group 33-40 on the non-fertilized plots and in all replication groups on the fertilized plots, 50-406 is the high parent. On the fertilized plots the F_1 hybrid is not significantly different from the low parent in the first four replication groups but is intermediate in group 33-40. Thus, for potassium, the dominance relationship and the inbred which is the low parent in the cross both vary with the fertilizer treatment and the replication group.

Variances

Analyses of variance of within-plot variances

The mean squares and F values from the analyses of variance of the within-plot variances are shown in Table 7. The sums of squares for populations and the interactions with populations are broken down into their component parts because the differences between geneities are of interest. The F values for popu-

Table 6.—Means of Logarithms of Parts per 100,000 of Sodium and Potassium for the Interaction of Replication Groups x Populations x Treatments.

Character and Population	Treatment and Replication Group									
	Non-fertilized					Fertilized				
	1-8	9-16	17-24	25-32	33-40	1-8	9-16	17-24	25-32	33-40
Sodium										
A54-1	1.0964	1.1126	1.2291	1.4450	1.6417	1.4420	1.4600	1.5112	1.6764	1.8675
A54-1, BB	1.0560	1.0612	1.1944	1.4398	1.5708	1.5357	1.3604	1.4734	1.7675	1.8500
50-406, BB	0.8892	0.9974	1.1246	1.3242	1.5244	1.1950	1.2640	1.2933	1.5399	1.6962
50-406	0.8706	0.9634	1.1286	1.3154	1.4652	1.2940	1.2874	1.4160	1.5944	1.6809
F_1 hybrid	0.9106	0.9413	1.1371	1.3452	1.5600	1.2422	1.2661	1.3618	1.6210	1.7450
52-307	1.1229	1.1210	1.2614	1.4526	1.6478	1.4108	1.4202	1.4630	1.7253	1.8442
Potassium										
A54-1	2.0682	2.0446	2.0473	2.1066	2.1168	2.1126	2.0588	2.0745	2.1173	2.1927
A54 1, BB	2.0235	2.0310	2.0319	2.0626	2.0936	2.0919	2.0794	2.0415	2.1100	2.1760
50-406, BB	1.9647	1.9854	1.9630	1.9813	2.0454	2.0199	2.0282	2.0137	2.0120	2.1167
50-406	1.9759	1.9890	1.9606	2.0291	2.0803	2.0809	2.0758	2.0593	2.0759	2.1134
F_1 hybrid	1.9847	1.9626	1.9472	1.9926	1.9979	2.0120	1.9957	1.9834	1.9745	2.0399
52-307	2.0364	2.0076	2.0057	2.0521	2.0067	1.9989	1.9971	1.9772	1.9886	2.0040

Table 7.—Analyses of Variance of Within-Plot Variances for Logarithms of Parts per 100,000 of Sodium and Potassium.

Source of Variation	Smaller Mean Square	Mean Square		F Value[2]			Tabular F			
							Sodium		Potassium	
		Sodium	Potassium	D.F.[1]	Sodium	Potassium	5%	1%	5%	1%
Replications		0.00077611	0.00003382	39	1.65f	2.16f	1.45	1.69	1.45	1.69
Populations		0.01720056	0.00031421	5	36.57f	15.52a	2.26	3.11	2.26	3.11
Geneities		0.06639155	0.00144698	1	173.92g	53.53b	4.10	7.35	4.10	7.35
Heterogeneous		0.00869156	0.00002621	2	10.80h	1.21c	3.11	4.88	3.11	4.88
Homogeneous		0.00111407	0.00003582	2	6.16i	2.31d	3.11	4.88	3.11	4.88
Treatments		0.01489786	0.00000476	1	16.60e	e	4.10	7.35	4.10	7.35
R x P	a	0.00038658	0.00002025	195	f	1.30f	1.26	1.39	1.26	1.39
R x G	b	0.00040364	0.00002703	39	1.06g	1.73f	1.71	2.14	1.45	1.69
R x He	c	0.00059104	0.00002160	78	h	1.38f	1.45	1.70	1.35	1.53
R x Ho	d	0.00017360	0.00001552	78	i	f	1.45	1.70	1.35	1.53
R x T	e	0.00089727	0.00002201	39	1.91f	1.41f	1.45	1.69	1.45	1.69
P x T		0.00079545	0.00003225	5	1.69f	2.06f	2.26	3.11	2.26	3.11
G x T		0.00085478	0.00001413	1	2.24g	f	4.10	7.35	3.89	6.76
He x T		0.00065358	0.00001830	2	h	1.17f	3.11	4.88	3.04	4.71
Ho x T		0.00090766	0.00005527	2	5.02i	3.54f	3.11	4.88	3.04	4.71
R x P x T	f	0.00047040	0.00001563	195						
R x G x T	g	0.00038174	0.00001609	39						
R x He x T	h	0.00080442	0.00001568	78						
R x Ho x T	i	0.00018071	0.00001535	78						

1. D.F. designates degree of freedom.
2. The letter after the F value indicates the error mean square used in calculating F.

lations are significant at the one percent level for both sodium and potassium. F values for all three components of the population sum of squares for sodium are significant at the one percent level. The only significant component of the population sum of squares for potassium is that for geneities. This indicates that for potassium the only differences between population variances are due to the residual variance present in the heterogeneous populations.

Significant differences between treatments are found for sodium but not for potassium. For replications the F value for sodium exceeds the five percent level while that for potassium exceeds the one percent level.

The R x P interaction for sodium is not significant. The R x P interaction for potassium is significant at the five percent level. A breakdown of this interaction reveals that the R x G interaction is significant at the one percent level, the R x He interaction is significant at the five percent level, while the R x Ho interaction is not significant. The R x T interaction for sodium is significant at the one percent level while that for potassium is not significant.

While the P x T interaction is not significant for either sodium or potassium, a breakdown of this sum of squares for sodium reveals that the Ho x T interaction is significant at the one percent level. In the potassium data the Ho x T interaction is significant at five percent level. Neither of the other components of the P x T interaction is significant for either character.

The R x P x T interaction is broken down into its components because the R x He x T interaction in the sodium data is significantly larger than either of the other components. There is no significant difference between the components of the R x P x T interaction in the potassium data.

Main effects

The mean within-plot obtained and residual variances for populations are shown in Table 8. The F_1 hybrid variance was subtracted from each of the other variances to obtain the residual variances. The significance of the residual variances is tested by using the F_1 hybrid variance as the lesser mean square and each of the obtained variances as the dividend in the F test. All the residual variances from the sodium data are significant at the one percent level. This result might seem to indicate genetic segregation within the inbred lines. If the inbred lines are segregating the variance of the F_1 should also be increased. Furthermore, no evidence of genetic segregation within the inbred lines is found in the analysis of the frequency distributions.

Table 8.—Mean Within-Plot Obtained and Residual Variances of Logarithms of Parts per 100,000 of Sodium and Potassium for Populations.

Population	Sodium		Potassium	
	Obtained	Residual	Obtained	Residual
A54-1	0.043171	0.030259	0.008309	0.004220
A54-1, BB	0.048346	0.035434	0.008079	0.003990
50-406, BB	0.028270*	0.015358	0.007223*	0.003134
50-406	0.020338*	0.007426	0.005165	0.001076
F_1 hybrid	0.012912		0.004089	
52-307	0.015972*	0.003060	0.003938*	-0.000151

Starred (*) obtained variances have 559 degrees of freedom; all others have 560. Tabular F for comparison between variances is 1.16 at the five percent level and 1.24 at the one percent level.

Of the residual variances obtained from the potassium data, those from the heterogeneous populations are all significant at the one percent level, that from 50-406 barely exceeds the one percent level, while that from 52-307 is not significantly different from zero.

First-order interactions

The mean within-plot obtained and residual variances for the interaction of populations x treatments are shown in Table 9. The F values for testing the significance of the residual variances are all significant at the one percent level for the heterogeneous populations. For sodium the residual variances for 50-406 are significant at the one percent level, but the residual variance for 52-307 is significant only on the non-fertilized plots. For potassium the residual variances for 50-406 are not different from zero. The obtained variance for 52-307 on the fertilized plots is significantly less than that for the F_1 hybrid. The residual variance for 52-307 on the non-fertilized plots is not considered since it was found that the frequency distribution for this population-treatment combination follows a normal curve on the arithmetic rather than the logarithmic scale.

Other comparisons of interest in Table 9 are A54-1 versus A54-1, BB and 50-406 versus 50-406, BB. On the fertilized plots the mean within-plot variances for A54-1 and A54-1, BB are not significantly different for either character. On the non-fertilized plots the F value for comparing A54-1 and A54--1, BB exceeds the five percent point for sodium while for potassium the variance for A54-1 is slightly higher, although not significantly so, than that for A54-1, BB. The F values for comparison of 50-406,

Table 9—Mean Within-Plot and Residual Variances of Logarithms of Parts per 100,000 of Sodium and Potassium for the Interaction of Populations and Treatments.

Treatment and Population	Sodium		Potassium	
	Obtained	Residual	Obtained	Residual
Fertilization				
A54-1	0.051484	0.033719	0.008035	0.003598
A54-1, BB	0.051962	0.034197	0.008543	0.004106
50-406, BB	0.037057	0.019292	0.007845	0.003408
50-406	0.027592	0.009827	0.005631	0.001194
F_1 Hybrid	0.017765		0.004437	
52-307	0.016575*	-0.001190	0.002909*	-0.001528
No fertilization				
A54-1	0.034858	0.026798	0.008582	0.004841
A54-1, BB	0.044729	0.036669	0.007615	0.003874
50-406, BB	0.019482*	0.011422	0.006600*	0.002859
50-406	0.013083*	0.005023	0.004700*	0.000959
F_1 Hybrid	0.008060		0.003741	
52-307	0.015369	0.007309	0.004968	0.001227

Starred variances have 279 degrees of freedom; all others have 280 degrees of freedom. Tabular F for comparison between variances is 1.26 at the 5% level and 1.39 at the 1% level.

BB with 50-406 on the fertilized plots approach the one percent level for both sodium and potassium, while on the non-fertilized plots the F values for comparisons between these populations are significant at the one percent level.

Since the residual variances for the heterogeneous populations are significantly different from zero, the frequency distributions were studied to determine the relative merits of the various population-treatment combinations as sources for selection of individuals high and low in sodium and/or potassium.

Frequency Distributions

Since the analysis of frequency distributions by the methods outlined by Powers, Robertson, and Clark (11), Powers, Robertson, and Remmenga (12), and Powers, et al., (13) depends upon the environmental variability being normally distributed, chi-square was used to test the fit of the frequency distributions obtained from the homogeneous populations to a normal curve. For sodium none of the homogeneous populations were distributed normally. When the data are transformed to logarithms, both the homogeneous and heterogeneous populations give satisfactory fits to a normal curve. Therefore, the transformed data were used in the statistical analyses.

For the potassium data only population 52-307 fits a normal curve satisfactorily when the original data are tested. When the data are transformed to logarithms, only populations 52-307 and 50-406 on the non-fertilized plots give significant chi-square values. For population 52-307 on the fertilized plots the test of whether the data are following the normal curve on the arithmetic or logarithmic scale is not discriminatory since neither chi-square value is significant. The P value for fit of the transformed data to the normal curve is 0.70-0.50, while that for fit of the untransformed data to the normal curve is 0.20-0.10. Similar results were reported by Powers (8). Statistical analyses of the potassium data were done on the transformed data. In the analysis of the individual frequency distributions, 52-307 on the non-fertilized plots was omitted since the data are following the normal curve on the arithmetic scale rather than on the logarithmic scale.

In order to estimate the environmental frequency distributions for the heterogeneous populations it is necessary to know whether the means and variances of the homogeneous populations are associated. Since there is no significant association between the mean within-plot variances and the means, for either sodium or potassium, the F_1 hybrid variance was used in the preceding section to test for the existence of residual variances. The variances to be used in analysing the frequency distributions are the total within-population and treatment variances. Thus it is necessary to examine the association of these variances with the corresponding means. The means and total within-population and treatment variances for the transformed data from the homogeneous populations are shown in Table 10. Regression of the means on the variances in the sodium data accounts for 85.21 percent of the variation among the variances. The m value of -0.0749 is different from zero with odds greater than 99:1 as measured by the t test. . Thus even though there is no significant association between the means and the mean within-plot variances, there is a significant association between the means and the total within-population and treatment variances. Because of this relationship, the regression of the means on the variances is used to predict the environmental variances following the methods outlined by Powers, Robertson, and Remmenga (12). The only difference between the total within-plot variances and the total within-population and treatment variances is the addition of the appropriate among-replications variance to each within-plot variance. Hence, the difference between the regression of the means on the mean within-plot variances and the regression of the means on the total within-population and

Table 10.—Means and Total Within Population and Treatment Variances for Logarithms of Parts per 100,000 of Sodium and Potassium for the Interaction of Homogeneous Populations and Treatments.

Treatment and Population	Sodium		Potassium	
	Mean	Variance	Mean	Variance
Fertilized				
50-406	1.4545	0.060825	2.0811	0.006412
F_1 Hybrid	1.4472	0.065401	2.0011	0.006138
52-307	1.5727	0.055824*	1.9931	0.003655*
Non-fertilized				
50-406	1.1487	0.079529*	2.0070	0.007627*
F_1 Hybrid	1.1788	0.092901	1.9770	0.004547
52-307	1.3212	0.073846	2.0217	0.006275

Starred (*) variances have 318 degrees of freedom; all others have 319.

treatment variances must be caused by differences in the among-replications variances for the different population-treatment combinations.

The question arises as to the justification for using this type of association in predicting the environmental variances for the heterogeneous populations, since the occurrence of a negative association between means and variances is seldom reported. The criteria of a high percentage of the variability accounted for by regression and a significant m value already have been shown to hold for the means and variances of the homogeneous populations. Examination of the replication group means and variances in the heterogeneous populations reveals a negative environmental relationship between these means and variances in that part of the distribution in which the mean falls. Thus the use of a negative regression to predict environmental variances is justified.

Examination of the regression of means on total within-population and treatment variances for the potassium data reveals that only 20.06 percent of the variation among the variances is accounted for by regression and that the m value is not significantly different from zero. Therefore the F_1 hybrid variance is used as an estimate for the environmental variance.

The logarithmic means, obtained and calculated frequency distributions, and the numbers and proportions of individuals genetically low and high in sodium and potassium are shown in Tables 11-14. Examination of these tables reveals that the heterogeneous populations each fall into three distinct classes as regards the relationship of the obtained and calculated fre-

Table 11.—Obtained and Calculated Frequency Distributions[1], Means of Data Transformed to Logarithms, and Numbers and Proportions of Individuals Genetically High and Low in Sodium, Fertilized Plots.

Population & Distribution	Mean	Upper Class Limits in Parts per 100,000																				Total Genetically	
		0 to 10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	160	170	180	190	Over 190	Low	High
A54-1	1.5914																						
Obtained		7	48	59	47	34	34	32	17	10	9	8	8	1	3	2					1	55	91
Calculated		2	32	67	66	51	35	23	15	10	6	4	3	2	1	1	1	1				34	67
Difference		5	16					9	2		3	4	5	-1	2	1	-1	-1			1	21	24
Proportion		0.71	0.33					0.28	0.12		0.33	0.50	0.62		0.67	0.50					1.00	0.38	0.26
A54-1, BB	1.5974																						
Obtained		9	55	40	48	45	29	24	20	11	9	10	4	7	5	2					2	64	70
Calculated		2	30	65	66	52	36	25	15	10	6	4	3	2	1	1	1	1				32	44
Difference		7	25						5	1	3	6	1	5	4	1	-1	-1			2	32	26
Proportion		0.78	0.45						0.25	0.09	0.33	0.60	0.25	0.71	0.80	0.50					1.00	0.50	0.37
50-406, BB	1.3977																						
Obtained		33	93	73	44	25	13	11	8	7	7	5	1									33	39
Calculated		21	93	84	53	29	17	10	5	3	2	1	1	1								21	23
Difference		12						1	3	4	5	4		-1								12	16
Proportion		0.36						0.09	0.38	0.57	0.71	0.80										0.36	0.41
50-406	1.4545																						
Obtained		10	79	90	54	29	25	10	13	6	1	1	2										
Calculated		12	76	84	58	36	21	13	7	5	3	2	3										
F_1 Hybrid	1.4472																						
Obtained		11	95	82	40	35	21	19	5	2	4	1	2	2		1							
Calculated		12	78	85	58	36	20	12	7	4	3	2	1	1	1								
52-307	1.5727																						
Obtained		3	38	74	74	45	18	21	9	10	15	7	3	2	1								
Calculated		2	37	70	67	49	34	22	14	9	6	3	2	2	1	2							

[1] The calculated frequency distributions were obtained by using the means of the transformed data, the standard errors of a single determination computed from the variances found in Table 10, and Pearson's (5) tables of the normal probability integral.

Table 12.—Obtained and Calculated Frequency Distributions[1], Means of Data Transformed to Logarithms, and Numbers and Proportions of Individuals

Table 12.—Obtained and Calculated Frequency Distributions[1], Means of Data Transformed to Logarithms, and Numbers and Proportions of Individuals Genetically High and Low in Sodium, Non-Fertilized Plots.

Population & Distribution	Mean	Upper Class Limits in Parts per 100,000															Total Genetically	
		0 to 10	20	30	40	50	60	70	80	90	100	110	120	130	140	Over 140	Low	High
A54-1	1.3050																	
Obtained		49	126	70	20	17	10	9	4	5	2	2	1		2	3	175	28
Calculated		43	116	77	40	20	11	6	3	2	1	1					159	13
Difference		6	10					3	1	3	1	1	1		2	3	16	15
Proportion		0.12	0.08					0.33	0.25	0.60	0.50	0.50	1.00		1.00	1.00	0.09	0.54
A54-1, BB	1.2645																	
Obtained		72	116	50	30	14	13	6	6	6		2	1		3	1	72	38
Calculated		55	122	72	35	17	8	5	3	1	1	1					55	19
Difference		17					5	1	3	5	-1	1	1		3	1	17	19
Proportion		0.24					0.38	0.17	0.50	0.83		0.50	1.00		1.00	1.00	0.24	0.50
50-406, BB	1.1720																	
Obtained		101	118	61	13	12	3	2	2		3	4	1				101	27
Calculated		89	126	59	25	11	5	3	1	1							89	21
Difference		12				1	-2	-1	1	-1	3	4	1				12	6
Proportion		0.12				0.08			0.50		1.00	1.00	1.00				0.12	0.22
50-406	1.1487																	
Obtained		99	126	57	19	9	2	3	1	3		1						
Calculated		100	121	54	23	10	5	3	1	1	1		1					
F_1 Hybrid	1.1788																	
Obtained		90	131	46	19	14	7	3	2	2	1	5						
Calculated		84	130	60	25	11	5	2	1	1		1						
52-307	1.3212																	
Obtained		44	125	62	34	26	13	7	2	1	2		2	2				
Calculated		38	113	78	42	22	12	6	3	2	1	1	1	1				

[1] The calculated frequency distributions were obtained by using the means of the transformed data, the standard errors of a single determination computed from the variances found in Table 10, and Pearson's (5) tables of the normal probability integral.

Table 13.—Obtained and Calculated Frequency Distributions[1], Means of Data Transformed to Logarithms, and Numbers and Proportions of Individuals Genetically High and Low in Potassium, Fertilized Plots.

Population & Distribution	Mean	Upper Class Limits in Parts per 100,000																				Total Genetically	
		0 to 60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	Over 240	Low	High
A54-1	2.1112																						
Obtained			2	5	14	27	42	47	31	31	32	22	16	22	10	9	4	2	1	2	1	90	51
Calculated				1	6	18	35	49	55	52	39	28	17	10	5	3	1	1				60	20
Difference			2	4	8	9	7							12	5	6	3	1	1	2	1	30	31
Proportion			1.00	0.80	0.57	0.33	0.17							0.55	0.50	0.67	0.75	0.50	1.00	1.00	1.00	0.33	0.61
A54-1, BB	2.0998																						
Obtained			3	6	20	28	52	40	35	22	26	25	19	19	11	9	1	3		1		109	88
Calculated				2	8	23	40	54	56	48	36	24	14	8	4	2	1					73	53
Difference			3	4	12	5	12					1	5	11	7	7		3		1		36	35
Proportion			1.00	0.67	0.60	0.18	0.23					0.04	0.26	0.58	0.64	0.78		1.00		1.00		0.33	0.40
50-406, BB	2.0381																						
Obtained		3	7	19	37	52	67	34	31	17	17	13	7	8	2	3	2			1		66	53
Calculated			2	12	32	54	65	50	44	26	14	7	3	1	1							46	26
Difference		3	5	7	5						3	6	4	7	1	3	2			1		20	27
Proportion		1.00	0.71	0.37	0.14						0.18	0.46	0.57	0.88	0.50	1.00	1.00			1.00		0.30	0.51
50-406	2.0811																						
Obtained			1	3	11	30	61	69	41	43	24	16	7	7	4	2					1		
Calculated				3	13	31	50	60	55	43	29	18	10	5	2	1							
F_1 Hybrid	2.0011																						
Obtained		2	6	23	60	66	74	47	17	16	6	2	1										
Calculated			7	26	54	71	64	47	27	14	6	3	1										
52-307	1.9931																						
Obtained			5	15	72	87	79	42	15	3	2												
Calculated		1	8	31	59	73	63	42	24	11	5	2	1										

[1] The environmental standard error used in obtaining the calculated frequency distributions was 0.0783.

Table 14.—Obtained and Calculated Frequency Distributions[1], Means of Data Transformed to Logarithms, and Numbers and Proportions of Individuals

Table 14.—Obtained and Calculated Frequency Distributions[1], Means of Data Transformed to Logarithms, and Numbers and Proportions of Individuals Genetically High and Low in Potassium, Non-Fertilized Plots.

Population & Distribution	Mean	Upper Class Limits in Parts per 100,000																				Total Genetically	
		0 to 60	70	80	90	100	110	120	130	140	150	160	170	180	190	200	210	220	230	240	Over 240	Low	High
A54-1	2.0767																						
Obtained			4	13	23	28	53	57	32	25	27	21	17	10	5	2		1		1	1	40	85
Calculated				1	9	30	56	69	62	45	26	13	6	2	1							10	48
Difference			4	12	14						1	8	11	8	4	2		1		1	1	30	37
Proportion			1.00	0.92	0.61						0.04	0.38	0.65	0.80	0.80	1.00		1.00		1.00	1.00	0.75	0.44
A54-1, BB	2.0485																						
Obtained		1	4	12	29	50	71	46	38	25	11	15	7	5	2	2	1		1			46	33
Calculated				4	21	50	71	71	50	30	14	6	2	1								25	9
Difference		1	4	8	8							9	5	4	2	2	1		1			21	24
Proportion		1.00	1.00	0.67	0.28							0.60	0.71	0.80	1.00	1.00	1.00		1.00			0.46	0.73
50-406, BB	1.9880																						
Obtained		3	16	36	58	77	52	34	19	10	5	6	1	2	1							55	25
Calculated			5	28	66	84	68	40	19	7	2	1										33	10
Difference		3	11	8						3	3	5	1	2	1							22	15
Proportion		1.00	0.69	0.22						0.30	0.60	0.83	1.00	1.00	1.00							0.40	0.60
50-406	2.0070																						
Obtained			4	29	61	77	59	35	20	9	11	5	5	2	2			1					
Calculated			3	17	50	78	75	52	28	12	4	1											
F_1 Hybrid	1.9770																						
Obtained		2	4	33	82	93	57	31	6	8	3		1										
Calculated		1	7	35	74	85	64	33	14	5	2												
52-307[2]	2.0217																						
Obtained		5	2	11	34	49	92	64	35	21	6		1										

[1] The environmental standard error used in obtaining the calculated frequency distributions was 0.0674.

[2] No calculated frequency distribution is presented for 52-307 because the transformed data do not follow a normal distribution.

quency distributions. Powers, Robertson, and Clark, (11) designated as identifiable-genetically superior or inferior those individuals which fell into the classes of the obtained frequency for which no individuals were expected. In classes at the ends of the frequency distributions for which more individuals were obtained than expected, the number in excess of those expected was called the number of genetic deviates. A similar terminology is employed in this paper except that the terms genetically high and genetically low in sodium or potassium are used instead of genetically superior and inferior, because there is no unanimity of opinion among authorities as to the level of sodium or potassium which should be considered superior or inferior.

The significance of differences between the obtained and calculated condensed frequency distributions was tested by the methods described by Powers, Robertson, and Clark (11). The divisions used in condensing the frequency distributions are indicated by the vertical lines in Tables 11-14. Chi-square and P values obtained from this test are shown in Table 15. For sodium, only 52-307, fertilized, of the homogeneous populations has a chi-square which exceeds the five percent level and this value lies between the five and two percent points. These results are further evidence that the significant residual variances obtained for the inbreds are not caused by genetic segregation.

Table 15.—Chi-Square and P Values for Fit of Obtained Condensed Frequency Distributions to Expected Environmental Frequency Distributions.

Character and Population	Fertilized		Non-Fertilized	
	Chi-Square	P	Chi-Square	P
Sodium				
A54-1	13.7532	0.01 -0.001	9.8806	0.01 -0.001
A54-1, BB	24.4198	0.001-1-inf.	11.4510	0.01 -0.001
50-406, BB	8.2918	0.02 -0.01	2.3138	0.50 -0.30
50-406	0.2530	0.90 -0.80	0.2082	0.95 -0.90
F_1 hybrid	0.4258	0.90 -0.80	4.1580	0.20 -0.10
52-307	6.3342	0.05 -0.02	1.1024	0.70 -0.50
Potassium				
A54-1	28.4158	0.001-1-inf.	38.1160	0.001-1-inf.
A54-1, BB	31.6066	0.001-1-inf.	23.7680	0.001-1-inf.
50-406, BB	17.7190	0.001-1-inf.	14.5766	0.001-1-inf.
50-406	0.2700	0.90 -0.80	13.9034	0.001-1-inf.
F_1 hybrid	0.1667[1]	0.95 -0.90	0.9921[1]	0.70 -0.50
52-307[2]	18.6398	0.001-1-inf.		

[1] These are goodness-of-fit chi-squares; the rest are homogeneity chi-squares.

[2] 52-307, non-fertilized was not included because the transformed data were not following a normal curve.

All the heterogeneous populations except 50-406, BB on the non-fertilized plots show significant differences between the obtained and calculated frequency distributions if odds of 49:1 are accepted as significant.

All the chi-square values from the heterogeneous populations for potassium are significant with odds greater than 999:1. Of the homogeneous populations, only 50-406, non-fertilized, and 52-307, fertilized have significant chi-square values. Significant chi-square values would be expected from these population-treatment combinations since the obtained variance for 52-307, fertilized is significantly less than that of the F_1 hybrid, and since 50-406, non-fertilized, while fitting a normal curve better on the logarithmic than on the arithmetic scale, still deviates from expected with odds between 99:1 and 999:1.

Study of Tables 11 and 12 reveals that for sodium there are no identifiable genetically low individuals in any of the heterogeneous populations. On the fertilized plots (Table 11) there is one identifiable genetically high individual in population A54-1, two in A54-1, BB, and none in 50-406, BB. On the non-fertilized plots (Table 12) there are six identifiable genetically high individuals in A54-1, five in A54-1, BB, and eight in 50-406, BB. Thus a significant number of identifiable genetically high individuals was found in 50-406, BB even though the chi-square test of the condensed frequency distributions failed to reveal any difference between the obtained and calculated frequency distributions.

Tests of whether the numbers of genetic deviates are significantly different from zero were made by the method outlined by Powers, Robertson, and Clark (11). On the fertilized plots the number of high and low genetic deviates in populations A54-1 and A54-1, BB are different from zero with odds greater than 99:1. For 50-406, BB, the number of high genetic deviates is significant with odds greater than 99:1, while the number of low genetic deviates is significant with odds of 60:1. On the non-fertilized plots the numbers of high genetic deviates in populations A54-1 and A54-1, BB are different from zero with odds greater than 99:1. Only A54-1, BB has a significant number of low genetic deviates on the non-fertilized plots. Population 50-406, BB has no significant number of genetic deviates in either the high or low class on the non-fertilized plots.

The breeding behavior of the heterogeneous populations for potassium is almost identical on the two fertility levels. On the fertilized plots (Table 13) two identifiable genetically low individuals were found in A54-1; three in A54-1, BB; and three

in 50-406, BB. On the non-fertilized plots (Table 14) four identifiable genetically low individuals were found in A54-1; five in A54-1, BB; and three in 50-406, BB. Five identifiable genetically high individuals were found in A54-1; four in A54-1, BB; and six in 50-406, BB on the fertilized plots, while on the non-fertilized plots five were found in A54-1; six in A54-1, BB; and four in 50-406, BB. The numbers of high and low genetic deviates in each population on both fertility levels are different from zero with odds greater than 99:1.

Joint frequency distributions

The joint frequency distributions for sodium and potassium are of interest in determining the possibility of altering sodium and potassium content simultaneously. The four classes, low sodium-low potassium, low sodium-high potassium, high sodium-low potassium, and high sodium-high potassium, are of interest. The four classes are obtained by using the same breaking points as those used in the individual frequency distributions shown in Tables 11-14. The question being asked is: within the classes in which there are genetic deviates for one character, are there genetic deviates for both characters? To determine this, the obtained joint frequency distributions are compared with the joint environmental frequency distributions expected on the basis of independence. The obtained joint frequency distribution for A54-1 on the fertilized plots is shown in Table 16. Study of Tables 11 and 13 reveals that the breaking points are between 110 and 120 for low potassium and between 170 and 180 for high potassium.

For sodium the breaking points are between 20 and 30 and between 60 and 70. The solid black lines in Table 16 delineate the classes formed by using these breaking points. To obtain the number of individuals expected in each of the four classes, the percentage of the total expected in the low and high classes for sodium, as shown in Table 17, and the number of individuals expected in the low and high classes for potassium are used. The numbers of individuals expected for potassium are listed in Tables 13 and 14 under the columns total genetically low and high opposite the row heading calculated. The product of the number expected for potassium and percentage expected for sodium gives the number of individuals expected in a particular class of the joint frequency distribution.

The obtained and calculated numbers and the number and proportion of joint genetic deviates in each of the four classes of interest are shown in Table 18. The number of individuals obtained in the low sodium-low potassium class for A54-1 on

Table 16.—Joint Frequency Distribution of

Table 16.—Joint Frequency Distribution of Sodium and Potassium for A54-1, Fertilized Plots[1].

Potassium upper class limits in parts per 100,000	Sodium upper class limits in parts per 100,000																
	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150	150 & over	Total
Over																	
220						2					1	1					4
220											1			1			2
210								1	1			1			1		4
200				1	2	1			1	1	1	1			1		9
190			1	3		1	2	1		1			1				10
180			1	1	3	4	2	4	2	1	1	1		1		1	22
170		1	1	1		5	2	2		1	2			1			16
160		1	3	4	5	3	2	2	1			1					22
150		6	5	6	3	2	6	1	1			2					32
140		2	8	4	6	2	3	2	1	3							31
130		6	8	2	5	3	2	2	2		1						31
120	3	11	12	6	4	3	6	2									47
110	3	8	10	7	3	4	3		1	1	1	1					42
100	1	9	5	7	1	1	3										27
90		3	4	4	1	1	1										14
80		1	1		1	1				1							5
70				1		1											2
Total	7	48	59	47	34	34	32	17	10	9	8	8	1	3	2	1	320

[1] Solid lines delineate classes formed by break points used in analysis of individual frequency distributions.

Table 17.—Percent of Individuals Expected in Classes Having Genetic Deviates for Sodium Data.

Treatment and Population	Class	
	Low	High
Fertilized		
A54-1	10.38	20.90
A54-1, BB	9.85	14.01
50-406, BB	6.55	7.49
Non-fertilized		
A54-1	49.60	4.18
A54-1, BB	17.11	5.94
50-406, BB	27.76	6.94

the fertilized plots is 25. This number is obtained by adding the numbers in the lower left hand corner of Table 16. The other obtained numbers for A54-1 are the total of the number of individuals in the appropriate corner of Table 16. The calculated number, six, is computed by multiplying 10.38 (see Table 17), the percent expected on the basis of the environmental frequency distribution for sodium, by 60, the number expected on the basis of the environmental distribution for potassium (see Table 14). The other calculated numbers are obtained in a similar manner. The number of individuals opposite the row heading difference in Table 18 are the number of joint genetic deviates for each class.

Examination of Table 18 reveals that for the high-high class the proportion of joint genetic deviates varies from 0.83 to 0.91. Tests of significance of the number of joint genetic deviates by the method of Powers, Robertson, and Clark (11) show that all the numbers of high-high genetic deviates are different from zero with odds of at least 99:1. Thus the choice of a fertility level and a population in which to select for individuals high in both characters depends on the number of individuals falling in the high-high class. The fertilized plots have from two to four times as many individuals in the high-high class as do the non-fertilized plots. Within the fertilized plots, A54-1, BB has the largest number of individuals in this class. Thus A54-1, BB should be the most fruitful source of individuals high in both sodium and potassium. For the low-low class the numbers of genetic deviates are different from zero with odds of at least 99:1 for all population-treatment combinations except 50-406, BB on the fertilized plots. For 50-406, BB the odds are 35:1 that the number of low-low genetic deviates is different from zero. On the fertilized plots A54-1 and A54-1,

Table 18.—Obtained[1] and Calculated Numbers and Numbers and Proportions of Genetic Deviates for Certain Classes of the Joint Frequency Distributions for Sodium and Potassium.

Treatment, Population, and Distribution	Class			
	Low Na Low K	Low Na High K	High Na Low K	High Na High K
Fertilized				
A54-1				
Obtained	25	0	12	31
Calculated	6	2	13	4
Difference	19			27
Proportion	0.76			0.87
A54-1, BB				
Obtained	28	9	9	47
Calculated	7	5	10	7
Difference	21	4		40
Proportion	0.75	0.44		0.85
50-406, BB				
Obtained	8	2	5	21
Calculated	3	2	3	2
Difference	5		2	19
Proportion	0.62		0.40	0.90
Non fertilized				
A54-1				
Obtained	26	26	2	12
Calculated	5	24	0	2
Difference	21	2	2	10
Proportion	0.81	0.08	1.00	0.83
A54-1, BB				
Obtained	12	2	2	11
Calculated	4	2	1	1
Difference	8		1	10
Proportion	0.67		0.50	0.91
50-406, BB				
Obtained	20	2	2	10
Calculated	9	3	2	1
Difference	11			9
Proportion	0.55			0.90

[1] The obtained and calculated numbers are those obtained and calculated on the basis of the population of 320 individuals.

BB have the largest numbers and proportions of joint genetic deviates in the low-low class, while on the non-fertilized plots A54-1 has the largest number and proportion of low-low genetic deviates. Examination of Tables 11-14 reveals that the individuals in population 50-406, BB fall into lower classes of the frequency distributions than do those of either A54-1 or A54-1, BB. Thus if the plant breeder wanted extremely low sodium and potassium, he probably would want to work within population 50-406, BB.

In the low sodium-high potassium class only A54-1, BB on the fertilized plots and A54-1 on the non-fertilized plots have joint genetic deviates. In neither case are the numbers of deviates significantly different from zero. For the high sodium-low potassium class, joint genetic deviates are found in 50-406, BB on the fertilized plots and in populations A54-1 and A54-1, BB on the non-fertilized plots. For A54-1 the odds are 21:1 that the number of deviates is different from zero. Only two high sodium-low potassium individuals are found in A54-1 on the non-fertilized plots, but these individuals are identifiable as genetically high in sodium and low in potassium since none were expected. Thus it may be possible to select for high sodium and low potassium simultaneously although it will be more difficult than selecting in either the high-high or the low-low classes.

Information on whether the individuals in the classes studied are there because of an environmental relationship or because of genetic reasons can be obtained by applying the chi-square test described by Powers (7) to the joint frequency distributions of the homogeneous populations. The only difference in the application of this test to the sodium and potassium data and the application made by Powers (7) is that in this case five classes, low sodium-low potassium, low sodium-high potassium, high sodium-low potassium, high sodium-high potassium, and the remainder of the joint frequency distribution are used instead of the two classes described by Powers (7). The classes with few individuals are combined so that at least five individuals are expected in each. The expected values are computed by using the mean and standard error of the transformed data for both sodium and potassium except for 52-307 on the nonfertilized plots. Since the potassium data from this population-treatment combination are following the normal curve on the arithmetic scale rather than on the logarithmic scale, the mean and standard error of the untransformed data are used to obtain the number expected in each class for potassium. The mean and standard error of the transformed data are used to obtain the percent expected for sodium. The chi-square and P values from this test are shown in Table 19. None of the chi-square values for either 52-307 or the F_1 hybrid exceeds the five percent level. For 50-406 the chi-square values are significant at the 0.001 probability level on both fertility levels. Since more individuals are obtained in the low-low and high-high classes than are expected, the relationship indicated by the significant chi-square values is positive. Since no evidence of genetic segregation was found for either character in the analysis of the individual fre-

quency distributions for 50-406, this result in the joint frequency distribution indicates an interaction of genotype and environment such that some genotypes show a positive environmental relationship between the two characters while other genotypes show no such relationship.

Table 19.—Chi-Square and P Values for Fit of Obtained Joint Frequency Distributions to Those Expected on the Basis of Independence of Sodium and Potassium for Five Classes of Interest in Homogenous Populations.

Population	Fertilized		Non-Fertilized	
	Chi-Square[1]	P	Chi-Square[1]	P
50-406	33.7268	0.001-1-inf.	53.0221	0.001-1-inf.
F_1 hybrid	6.3887	0.20 -0.10	9.2634	0.10 -0.05
52-307	4.9464	0.30 -0.20	8.8593	0.10 -0.05

[1] Degrees of freedom for chi-square are 4 in every case.

Discussion

Although Rorabaugh and Norman (14) reported that their tests of solubility of sucrose in salt solutions indicated that sodium, in combination with carbonate, chloride, and sulfate ions, was more harmful than potassium, the effect of the presence of sodium and/or potassium ions on the processing quality of sugar beets has not been determined. Neither has the exact role of sodium and potassium in plant metabolism been determined. Finkner and Bauserman (3), in a study of breeding behavior, concluded that sodium, per se, had little effect on sucrose content and that the negative relationship between sucrose and sodium could be easily broken. They stated that sodium content was of little value in the breeding program unless it is extremely important from the processing standpoint. Wood (15), and Wood, et al., (16) in a review of breeding work which has been done for chemical characters, reported significant decreases in sodium content as a result of selection. He stated that the value of reduction in sodium from the processing standpoint was not known. With respect to potassium he reported that differences between 16 varieties of sugar beets, while they might be statistically significant were too small to be of practical value. Doxtator and Calton (2), studying sodium and potassium contents of three varieties in two areas, found significant differences between varieties and areas but no significant interaction of varieties and areas. They reported differences between beets within varieties for both sodium and potassium. Doxtator and Bauserman (1) in limited breeding trials concluded that changing sodium content was

possible by simple breeding methods but that changing potassium content would be more difficult.

Study of the homogeneous populations indicates that low sodium content is dominant. This is true on both the fertilized and the non-fertilized plots. This result indicates that by using inbred lines in a breeding program it should be relatively easy to obtain a low-sodium hybrid. The potassium picture is more complicated since the cross studied shows dominance for low potassium on the high fertility level and heterosis for low potassium on the low fertility level. Furthermore the low parent on the high fertility level is the high parent on the low fertility level. The behavior of the F_1 hybrid might be explained by assuming that the ability to produce low potassium on the fertilized plots, found in 52-307, is dominant, while the ability to store less potassium on the non-fertilized plots than on the fertilized plots, found in 50-406, is also dominant. If such is the case, the F_1 hybrid should show heterosis for low potassium on the non-fertilized plots and it does.

The next aspect of breeding behavior to be examined is that of the variability in populations A54-1 and 50-406 after exposure to pollen from 22 other sources to form broad-base populations. Exposure of A54-1 to 22 other kinds of pollen had little effect on its variability in sodium and potassium contents, while exposure of 50-406 to 22 other kinds of pollen significantly increased its variability. This can be explained by assuming that A54-1 is heterozygous and heterogeneous enough to encompass the range of sodium and potassium contents found in the 22 other populations while assuming that the variability in 50-406, an inbred line, is limited to that due to environmental causes and that the increased variability of 50-406, BB is caused by increased genetic variability.

The analysis of frequency distributions provides most of the information concerning the potential breeding value of the heterogeneous populations. Study of the within plot variances shows that there is residual variance in the heterogeneous populations. The analysis of the frequency distributions reveals that selection for low sodium content can be more readily accomplished on the fertilized plots while selection for high sodium can be more readily accomplished on the non-fertilized plots. Comparisons of populations within treatments reveals that selection for low sodium should be more successful in population A54-1 since this population contains both a larger number and a larger proportion of genetic deviates than either of the other populations. Populations A54-1 and A54-1, BB are superior to

50-406, BB for selection of genetically high-sodium individuals since the genetic deviates fall into higher classes of the frequency distributions and since the numbers and proportions of genetic deviates are higher than in 50-406, BB.

Analysis of the frequency distributions for the potassium data reveals that the breeding behavior of the heterogeneous populations is essentially the same on both soil-fertility levels. Selection for low potassium should be most effective in population 50-406, BB while selection for high potassium should be most effective in A54-1.

Study of the joint frequency distributions for sodium and potassium indicates that it should be relatively easy to select individuals genetically high in both sodium and potassium or genetically low in both sodium and potassium. Selection of individuals genetically high in sodium and low in potassium or low in sodium and high in potassium would be more difficult. Thus a positive relation between sodium and potassium is indicated. A positive correlation between sodium and potassium was reported by Doxtator and Calton (2). It should be remembered in interpreting the results of the analysis of the joint frequency distributions that the joint environmental frequency distributions were calculated on the basis of independence of environmental effects for sodium and potassium. It was found for 52-307 and the F_1 hybrid that this assumption is correct, but that there is a positive environmental relationship between sodium and potassium in population 50-406. Thus the joint genetic deviates may be in the class in which they fall because of linkage, pleiotropy, or an interaction of genotype and environment.

From a practical sugar beet-breeding standpoint, the relation between sodium and potassium in the beet may not be as important as the relation between these characters and sucrose content, weight of root, and nitrogen content of the root. These characters have been studied and reported on (4, 13), but their interrelation with sodium and potassium have not yet been fully evaluated.

Summary

1. Studies of the variability and breeding behavior of sodium and potassium contents in the sugar beet root were made for six populations at two different levels of soil fertility.

2. It was found that low sodium was dominant for both fertility levels, but that low potassium was dominant on the fertilized plots while heterosis for low potassium was exhibited on the non-fertilized plots.

3. An interaction of populations and treatments such that the high-potassium parent on the fertilized plots was the low parent on the non-fertilized plots was found.

4. Analysis of the within-plot variances showed that all the heterogeneous populations contained significant residual variances and that it should be possible to alter their sodium and/or potassium content.

5. A significant negative relation between the means and the total within-population and treatment variances was found in the sodium data.

6. Analysis of the frequency distributions revealed that the sodium data were following a logarithmic rather than an arithmetic scale. The potassium data except for 52-307 on the non-fertilized plots were also following a logarithmic scale. The data from 52-307, non-fertilized, were following an arithmetic scale more closely than a logarithmic. Both the sodium and the potassium data were transformed to logarithms for statistical analyses.

7. Analysis of the individual frequency distributions revealed that it should be possible to select individuals genetically high or low in sodium content. Selection for low sodium could be done more easily on the fertilized plots, while selection for high sodium could be more readily accomplished on the non-fertilized plots.

8. Selection for high or low potassium could be accomplished with equal facility at either soil fertility level. Study of the differences between populations revealed that 50-406, BB would be the most desirable population from which to select for low potassium and that A54-1 would be the most desirable for selection for high potassium.

9. Analysis of the joint frequency distributions revealed that it would be possible to select readily for individuals high or low in both sodium and potassium but that it would be much more difficult to select for individuals high in sodium and low in potassium or high in potassium and low in sodium.

10. Analysis of the joint frequency distributions of the homogeneous populations revealed no environmental relation between sodium and potassium for 52-307 and the F_1 hybrid but a significant environmental relation between sodium and potassium was found for 50-406.

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Incidence of Rhizoctonia Crown Rot of Sugar Beets in Irrigated Crop Rotation[1]

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Introduction

The incidence of sugar beet crown and root rot in crop rotation has been reported by several investigators (1, 3, 7, 8, 9)[3]. These reports cited above are based for the most part on surveys and are not the result of planned rotation experiments to observe the specific diseases associated with crop rotations. Results on the incidence of Rhizoctonia crown and root rot reported in our paper and elsewhere confound to some extent the effect of the preceding crop and time between sugar beet crops on the incidence of Rhizoctonia induced crown and root rot.

Crown and root rot, an important disease of half-grown or nearly mature sugar beets (*Beta vulgaris* L.) in western Nebraska and other areas is caused by *Rhizoctonia solani* Kuhn, (*Pellicularia filamentosa* (Pat.) Rogers). *R. solani* has a wide host range and comprises numerous parasitic strains. This characteristic of the pathogen and the fact that *R. solani* survives in the soil influences the use of control measures, such as crop rotation. The effects of crop rotations on crown rot of sugar beets at the Scotts Bluff Experiment Station, Mitchell, Nebraska, were determined for the years 1933 and 1934 and for the period 1946 to 1949. More recently experiments on cross inoculations were made with *R. solani* isolates to ascertain the possible relationship of parasitic strains to the effectiveness of crop rotation in controlling the disease. Data from crop rotation and cross inoculation experiments are incorporated in this paper.

Materials and Methods

The rotation plots used in this investigation are located at the Scotts Bluff Experiment Station, Mitchell, Nebraska, on the North Platte reclamation project in western Nebraska. The elevation of the Station is approximately 4000 feet. These rotations were inaugurated in 1912 and deal with various methods of utilizing irrigated land. They were carried out as originally planned from 1912 to 1941. During this 30-year period the

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[3] Numbers in parentheses refer to literature cited unless indicated otherwise.

rotation experiments dealt primarily with the use of farm manure and legumes (alfalfa and sweet clover) for maintaining soil productivity.

A new rotation put into effect from 1942 to 1949, included manure, mineral fertilizer (chiefly ammonium sulphate and treble superphosphate), alfalfa as green manure, and sweet clover as a pasture and green manure. Barley replaced oats. The other crops in the rotation were alfalfa, sweet clover, sugar beets, potatoes, and field beans (Table 1).

Table 1.—New Rotation Numbers, Crop Sequences and Fertilization at the Scotts Bluff Experiment Station, Mitchell, Nebraska.

New Rotation No.	Crop Sequence and Fertilization[1]
2	beets
2D	beets (NP)
20	beets, potatoes
20C	beets (NP^2), potatoes (NP^2)
21	beets (M), potatoes
33	beets, barley-sweet clover, potatoes
33B	beets (M), barley-sweet clover, potatoes
35	beets, barley, potatoes
35B	beets (M), barley, potatoes
35C	beets (NP^2), barley, potatoes (NP^2)
41	beets, barley-alfalfa, alfalfa, potatoes
41B	beets (M), barley-alfalfa, alfalfa, potatoes
41C	beets (NP), barley-alfalfa, alfalfa, potatoes (P)
43B	beets (M), barley, beans, potatoes
43C	beets (NP^2), barley, beans, potatoes (NP^2)
49	beets, barley-sweet clover, sweet clover pasture, potatoes
49C	beets (NP), barley-sweet clover, sweet clover pasture, potatoes
49D	beets (NP), barley-sweet clover, sweet clover cut, potatoes (P)
63	beets, barley-alfalfa, alfalfa (3 yrs.), potatoes
63B	beets (M), barley-alfaifa, alfalfa (3 yrs.), potatoes
63C	beets (NP), barley-alfalfa, alfalfa (3 yrs.), potatoes (P)
66B	beets (M), barley-alfalfa, alfalfa (2 yrs.), potatoes, beans
66C	beets (NP), barley-alfalfa, alfalfa (2 yrs.), potatoes (P), beans

[1] (M) farm manure applied at rate of 12 tons per acre.
(NP) 102 pounds of nitrogen and 56 pounds of P_2O_5 per acre.
(NP^2) 51 pounds of nitrogen and 56 pounds of P_2O_5 per acre.
(P) 56 pounds of P_2O_5 per acre.
Previous to 1945, the application of nitrogen amounted to 20 pounds per acre.

The land had not been cultivated prior to the establishment of the Station in 1910. The soil of the experimental area is Tripp very fine sandy loam, a typical soil of the extensive areas of the terraces north of the North Platte River. It is readily permeable to water and well drained. Each crop in the rotation was grown each year; there were as many plots for each rotation as there were years in the cycle of the rotation. As far as possible, the same cultural treatments were given to all plots of the same

crop. All plots in sugar beets were planted each year for the 1946-1949 period to the variety GW 526; a variety very susceptible to crown rot. The variety used in 1933 and 1934 is not known. It may or may not have been from seed grown in Europe.

Plots 2 and 2D were each one-eighth acre in size. All others were one-fourth of an acre. The rows were 132 feet long. Crown rot data for 1933 and 1934 have been obtained from sugar beet root samples taken at random just prior to harvest. The method of sampling consisted of taking five samples of 20 beets each from 40 inside rows of each plot. Ten beets were taken from each 4 rows. Eight rows comprised one sample. The data for the 1946-1949 period were taken from six specific and uniformly distributed inside rows in each plot. Observations and data on the incidence of crown rot were made twice during the growing season and at harvest time. About 700 beets per plot were observed per season.

The modified toothpick method (10) of inoculation was employed in greenhouse and field trials to determine the host range of *R. solani* strains. Potatoes (variety Progress) and field corn (variety Nebr. 501-B) were inoculated in the first node. Field beans (variety U.I. 59) were inoculated in the tap root. The field corn, beans, and potatoes were about one month old at time of inoculation.

An attempt was made to determine the inoculum potential of soil from the rotation plots by planting seed in soil samples under greenhouse conditions. Adequate checks consisted of steam sterilized soil and normal greenhouse soil. Each soil sample was tested in duplicate in 6-inch clay pots in each of which 25 sheared sugar beet seed were planted. The air temperature of the greenhouse was 85° F.

Experimental Results

The percentage of diseased sugar beets in the rotations for 1933 and 1934, 22 and 23 years after the rotations were established, was present at a low level in rotations from 2 to 6 years in length as well as in the continuous sugar beet plot at this time. The maximum percentage was about 3 percent for any one period. A disease trend related to rotation length and type was not evident in 1933 and 1934.

The data for the 1946-1949 period indicated a definite trend in disease incidence (Table 2). The incidence of crown rot of sugar beets was the highest in the continuous cropping of sugar beets, rotations 2 and 2D; lowest in the four- and six-year rotations, rotations 41 through 66C. In general as the length of rotation decreased the incidence of crown rot increased.

Table 2.—Prevalence of Crown Rot of Sugar Beets in Irrigated Rotations in 1946 to 1949 at the Scotts Bluff Experiment Station, Mitchell, Nebraska.

Rotation No.[1]	Percent of Crown Rot				
	1946[2]	1947	1948	1949	Average
2	53	80	62	56	63
2D	70	64	48	40	56
20		27	37	33	32
20C		13	28	14	18
21		4	34	28	22
33		12	7	15	11
33B		7	9	13	10
35		21	11	12	15
35B		8	15	10	11
35C		8	10	8	9
41		7	18	17	14
41B		4	11	4	6
41C		6	12	14	11
43B		7	9	12	9
43C		7	12	5	8
49		2	8	15	8
49C		5	7	12	8
49D		9	11	14	11
63		13	3	17	11
63B		3	17	11	10
63C		5	14	12	10
66B		9	8	9	9
66C		14	14	18	15

[1] See Table 1 for description of rotations.

[2] Data not obtained for plots other than for 2 and 2D.

Considering all rotations, the application of either farm manure or commercial fertilizer decreased the Rhizoctonia disease (Table 3). In continuous sugar beet cropping commercial fertilizer decreased the percentage of crown rot from 63 to 56 for the four-year period, 1946-1949. In the two-year rotation for a three-year period, 1947-1949, the incidence of disease de-

Table 3.—Average Percent of Crown Rot of Sugar Beets for the Fertilization Treatments in Different Lengths of Rotation for the 1946-1949 Period.

Length Rotation in Years	Non-fertilized	Manure	(NP)
continuous	63		56
2	32	22	18
3	13	11	9
4	11	8	10
6	11	10	13
Average	28	13	21

creased from 32 to 22 and 18 with the application of farm manure and commercial fertilizer, respectively. There was little difference between the non-fertilized and fertilized plots in three-, four- and six-year rotations.

The effect of crop sequence on crown rot of beets was difficult to determine. The sugar beet crop followed potatoes in all rotations except 66B and 66C where it followed beans (Table 1). The incidence of crown rot was relatively low and about equal in all six-year rotations.

Under greenhouse conditions crown rot did not develop in sugar beets planted in soil from different rotations. A considerable amount of pre-emergence damping-off was noted. No clear-cut differences, however, could be detected either between different lengths of rotation or between different treatments within the same length rotation. The average percent loss in stand for the continuous cropping and two-, three-, four- and six-year rotation was 36, 16, 40, 28 and 32. The stand loss in normal greenhouse soil was 6 percent and when sterilized no loss in stand was realized.

The temperature of 85° F. is probably too high to occur naturally during spring conditions but this temperature is common later in the season when most of the crown rot appears. The experiment bears out the fact that the soils from the rotation plots contain damping-off microflora and under certain conditions this disease may occur. The information on damping-off in the greenhouse is of dubious value from the crown rot standpoint because *R. solani* strains that cause the damping-off phase may not necessarily be capable of causing the crown rot phase of more mature sugar beets (10). A stand loss due to damping-off in the rotations did not appear to be common under field conditions.

Cross inoculations with *R. solani* isolates were made to ascertain the reaction of crops grown in the rotations. Cultures 2, 5, 8, 13 and 20 were isolated from diseased sugar beet roots and Cultures 1 and 42 were obtained from potato stems and sugar beet soil (irrigation runoff water), respectively. In 1958 greenhouse experiments Cultures 1, 2, 8, 13, 20 and 42 were found to be nonpathogenic for field beans. Cultures 2 and 42 induced only a slight discoloration in the area of inoculation. This discolored area was slightly more extensive than for the other cultures but the isolates will be classified as non-pathogenic because the spread of discoloration during a three-month period may not be due to true infection. Each of the Cultures 2, 5 and 13 killed about 75 percent of potatoes inoculated; Cultures

1, 20 and 42 were nonpathogenic for potato. Cultures 2 and 8 induced severe crown rot symptoms of sugar beets and the other four cultures were classified as nonpathogenic using the toothpick inoculation method (10) or by soil infestation in the field.

In field experiments results similar to those reported for greenhouse tests were obtained with Cultures 1, 2 and 5. In 1958 field tests Culture 1 was nonpathogenic for field corn, potatoes and field beans. Cultures 2 and 5 were definitely pathogenic for potatoes causing 100 percent kill. These cultures caused slight discoloration in inoculated areas in field corn and were classified as nonpathogenic for field beans. In 1959 field tests the results of the 1958 tests were confirmed for Cultures 1 and 2. Culture 8 was pathogenic for potatoes but not for field beans or field corn.

Soil infestation in pots with Cultures 1 or 2 in greenhouse tests resulted in host reaction comparable to data reported previously. Neither Culture 1 nor 2 affected alfalfa, a crop which was not included in toothpick inoculation experiments. Culture 23, isolated from field bean roots, proved pathogenic only for field beans because no stand was obtained in soil infested with this culture.

Discussion

In considering the effects of crop rotations on the incidence of a soil-borne disease several aspects need to be taken into account. What is the host range of the pathogen with respect to crops grown in the rotation? What is the effect of the preceding crop on the disease? What is the effect of fertilization on the occurrence of the disease?

The length of rotation definitely affected the amount of crown rot of sugar beets. Sugar beet monoculture and two-year rotations with potatoes were favorable for *R. solani* survival and development. Two-year rotations with crops such as corn, beans or alfalfa substituted for potatoes would clarify the reason for disease incidence. In other words, *R. solani* may be capable of survival as a saprophyte in the soil for two years and decrease with time. This information would present a clearer picture of the effect of length of rotation on the disease.

An explanation for the effect of crop rotations on Rhizoctonia disease was sought by a determination of the parasitic capabilities of strains of *R. solani* on crops grown in the rotations. Isolates of this fungus from sugar beets were pathogenic for sugar beets and potatoes but not for field beans, alfalfa and field corn. An isolate from potato stems was nonpathogenic for field corn, field beans, potatoes and sugar beets. This nonpathogenicity of potato

stem isolates for sugar beets confirms LeClerg's work (6, 7). An isolate from field bean root was only pathogenic for field beans. A pigweed (*Amaranthus retroflexus* L.) isolate was slightly pathogenic for sugar beets. The number of isolates employed may not include the entire spectrum of parasitic capabilities represented by *R. solani* strains existant in western Nebraska. The data may be somewhat indicative of the host range of the races present, for Rhizoctonia root rot is not an economic problem on any of the crops grown under irrigation in western Nebraska other than sugar beets or potatoes.

With the many strains of *R. solani* existant a general statement as to its pathogenic capabilities may be incorrect. In 1934 LeClerg (6) had found that *R. solani* isolated from potato stems and tubers were nonpathogenic for sugar beets. The isolates from potatoes are generally considered as nonpathogenic for sugar beets (3, 6). For this reason potatoes have been suggested as the crop to precede sugar beets. More recently some of the *R. solani* isolates from potato stolons were found pathogenic for sugar beets (7). On the other hand our studies have indicated that *R. solani* isolates from sugar beets may or may not be parasitic for potatoes. Thus a crop rotation effect on the *R. solani* disease may result from host selection of pathogenic strains. Strains pathogenic for sugar beets or potatoes survive because they are able to parasitize these crops. Although exact data to prove this point are not available, our studies show that in a two-year rotation the incidence of *R. solani* crown rot is decreased considerably compared to continuous cropping of sugar beets. In 1938 Goss and Afanasiev (4) reported a similar decrease in Rhizoctonia black scurf of potatoes in the same rotations. The fact that tests of isolates of *R. solani* from potato tubers are nonpathogenic for sugar beets favors the idea of selective pressures. The longer rotations are the more effective in keeping the disease in check because field beans, alfalfa and sweet clover may not favor survival and development of *R. solani* strains pathogenic for sugar beets or potatoes.

Thus, in spite of the fact that many strains of *R. solani* exist, conditions in certain localities favor the survival of some strains. In western Nebraska *R. solani* may be prevalent on sugar beets and potatoes because strains pathogenic for these crops are favored by conditions present. It is quite probable that under certain conditions the *R. solani* strains pathogenic for either field beans, alfalfa, or sweet clover may not be important on either sugar beets or potatoes.

Surveys by LeClerg (7) of sugar beet and potato fields in Colorado, Wyoming, and Nebraska for the period 1936-1938

showed that sugar beets following potatoes were, in general, relatively free from Rhizoctonia root rot compared with sugar beets followed by sugar beets. There are reports (1, 8, 9) of Rhizoctonia root rot of sugar beets following potatoes. In these instances other crops besides the one just preceding sugar beets may need be considered. In the Scotts Bluff Experiment Station rotation plots the incidence of Rhizoctonia crown rot of sugar beets was low when preceded by potatoes in a four- or six-year rotation, but was high in the shorter rotations.

Discrepancies in field survey data from different areas may, in part, be attributed to variations in pathogenicity of *R. solani* isolates. In the Scotts Bluff Experiment Station rotations, 70 and 63 percent Rhizoctonia infection occurred in four years of continuous cropped potatoes and sugar beets, respectively, while only 26 and 32 percent occurred in a two-year rotation of potatoes and sugar beets (4).

An explanation of the incidence of Rhizoctonia disease requires more information than a statement on crop sequence. Addition of fertilizers to the soil decreased the disease both in potatoes and sugar beets (5). Knowledge of fertilization practice at the time interval between susceptible crops is needed. Coons (2) presents a general review of the Rhizoctonia disease of sugar beets and Kernkamp et al., (5) present a review of the many complex problems involved in Rhizoctonia diseases of plants. The many ramifications of the problem will not be repeated in this paper.

A comparison of the data obtained in 1933 and 1934 with that in 1946-1949 indicates a cumulative increase in Rhizoctonia crown rot over a period of time. In 1933 and 1934, however, the data relate to harvested roots. Sugar beets killed early in the season during these years were ignored. Therefore, the cumulative increase may be less important than the data indicated.

Summary

A study of the effects of irrigated crop rotations on *Rhizoctonia solani* crown and root rot of sugar beets was made at the Scotts Bluff Experiment Station, Mitchell, Nebraska. In addition to continuous cropping plots, four different lengths of rotations were involved as well as different fertilization and cropping practices.

There was a tendency for an increase in disease with a decrease in length of rotation. The amount of disease was favored by continuous cropping and by a two-year rotation. The host range of several strains of *R. solani* was studied to explain the

effects of crop rotation on the disease. Crown rot was present in the rotations 22 and 23 years after inauguration of the rotations but appeared more common 34 and 36 years after the program was started.

Fertilizer applications decreased crown rot in the two-year rotation and in 3 out of 4 years of continuous cropping. Commercial fertilizer or manure did not appear to affect the disease incidence in the longer rotations.

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Determination of Microorganisms in Sugar Products by the Millipore Method

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Molecular filter membranes, which are manufactured in this country and in Europe, have found recognized applications in laboratories for precise particle separation from liquids and gases. The successful use of the membranes for the separation of microorganisms from air and liquids is also well established. Due to the wide range of porosities offered by the manufacturers and the remarkably low variance in the stated pore size, it is possible to select a membrane which is capable of trapping certain organisms while allowing a maximum filter flow. These properties, combined with subsequent incubation, looked attractive for the microbiological analyses of sugar products. The apparent advantages visualized over the conventional plating methods were, principally:

1. Possibility of larger sample size.
2. Less equipment required.
3. Less time required for preparation of sample.
4. Less chance for contamination.
5. Shorter incubation time, as had already been experienced in water analyses.
6. Staining and counting the trapped organisms without incubation.

Some of these apparent advantages have been realized, in particular the first four. There has, however, been some doubt raised regarding the reliability of the membrane method. Such doubt was probably caused by the frequent lack of agreement between the membrane method aand the conventional plating methods. When such disagreement occurred there has frequently been an unfounded tendency to consider the plate method to be the correct one.

Older methods used for the enumeration of microorganisms in sugars were well established and approved by canners and bottlers. When the same methods were used by different laboratories good agreement in results could be expected on the same sample. The possibility of the presence of spoilage organisms, other than those found, was not always recognized or was overlooked. This attitude was not nearly as dangerous in the days of dry sugars only, as it is now when a very considerable portion of the sugar production is in the form of liquid sugars.

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Our present knowledge indicates that the organisms we should be mostly concerned with in liquid sugars are the yeasts and molds. Of the two, the yeasts appear to be the most troublesome.

Several investigators within the sugar, canning or bottling industries have expressed the opinion that the term yeast, as now used in standards for sugars, must be further clarified. Yeasts found in sugar products may be of several types with different growth requirements. Variations in growth requirements indicate a need for different means of detection and enumeration of specific yeast types.

The research department of the Spreckels Sugar Company has conducted a series of comparative tests for the enumeration of yeasts in liquid sugars by the filter membrane method and the ABCB method.

Two porosities of filter membranes were used, 0.45 μ and 0.80 μ. The membranes are manufactured and distributed by the Millipore Filter Corporation, Bedford, Massachusetts, under the trade name MF.

The ABCB method is that tentatively adopted by the American Bottlers of Carbonated Beverages.

The media used were:

ABCB method	Mycophil Agar with low pH (BBL)[2]
MF method	Mycophil Broth with low pH (BBL)[2]
or	Osmophilic Broth (de Whalley medium without agar) (1)[3]

Tests were run on sterile liquid sugars inoculated with pure cultures of various yeasts isolated from liquid sugars obtained from sources outside the company. These yeasts have not been positively identified and are designated herein as A, B, and C.

It was decided to run the tests on pure yeast cultures in order to avoid the necessity of identifying types and also to avoid the interference of molds. In most cases, except in very low counts or when gross contamination occurs, the yeasts found in liquid sugars are predominantly of one type. The use of pure cultures was only an extra precaution.

Tests were run on 20 grams dse (dry sugar equivalent) portions of liquid sugar. The ABCB method, which specifies the use of five grams dse on four plates, was run in four sets of four plates, each, for a total of 20 grams dse.

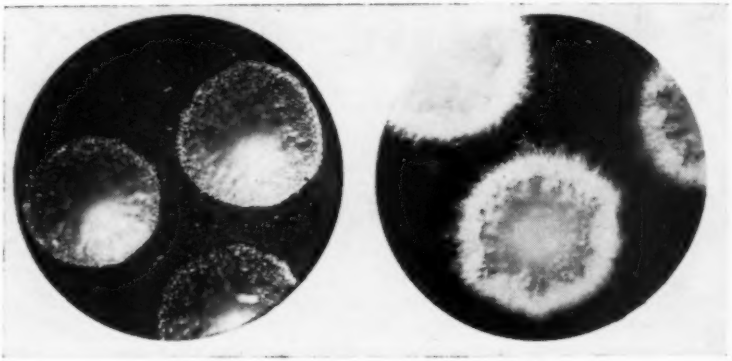
The period of incubation was three days in all cases at a temperature of 31° to 33° C. The conventional 100 mm ×

[2] Baltimore Biological Laboratory, Inc.
[3] Numbers in parentheses refer to literature cited.

15 mm glass Petri dishes were used in the ABCB method. Tops or bottoms of the 60 mm × 15 mm glass Petri dishes, covered during incubation with "Parafilm," were used in the MF method. The actual manipulation of the MF membrane method, such as filtration of sample, use of absorbent pad, etc. have been described by others (2) (3).

The yeast colonies were counted by 9X magnification, using a stereoscopic microscope with reflected light.

Yeast "B"



0.45μ 0.80μ

Figure 1.—Yeast colonies grown on 0.45 micron and 0.80 micron MF membranes (magnified from a field of 2mm diameter).

The yeast colonies grew to approximately the same size on all three media. Those grown on .80 μ membranes were generally more mature and better defined than those grown on either the .45 μ membrane or the agar medium. Figure 1 shows colonies of yeast B grown on .45 μ and .80 μ membranes, respectively. The better yeast definition on the .80 μ membrane may allow a shorter incubation time than was used in the present tests.[4]

Table 1 is a summary of results. The results obtained allow the following observations:

1. There is no significant difference between the results obtained by .45 μ and .80 μ membranes when the same medium is used. As mentioned above, the organisms are

[4] In previous tests conducted by the research department of Spreckels Sugar Company the possibility was indicated that the .80 μ membrane might permit the development of a higher number of organisms than the .45 μ membrane. The tests were run on samples containing a mixed microbiological flora, i.e., possibly more than one type of yeast or mold. Some of the yeasts may have been inhibited on the .45 μ membranes and therefore were incapable of developing in the same time period as those on the .80 μ membrane.

better defined on the .80 μ membrane, which also allows faster filtration of the sample. This latter feature may permit the use of larger sample size than 20 grams dse.

2. Yeasts A and B grow equally well on either of the nutrient broths. Results are comparable for all three methods except in one test where definitely lower results were obtained by the ABCB method.

3. Yeast C shows a definite preference for Mycophil broth (low sugar content) and its growth may possibly be inhibited on Mycophil agar (ABCB method) at least at high counts. It is definitely inhibited when grown on osmophilic broth (40% sugars).

Table 1.—Yeast Counts in Liquid Sugars Obtained by the Use of Three Methods. Twenty Grams dse Sample Size Was Used in All Tests. Results Are Calculated to 10 Grams dse.

	Mycophil Broth		Osmophil Broth		Mycophil Agar
	MF .45 μ	MF .80 μ	MF .45 μ	MF .80 μ	Plate ABCB
Yeast A	95	95	70	83	108
	115	86	90	81	95
	51	41	44	43	41
	21	15	27	28	10
	24	26	26	26	25
	63	68	62	63	62
	34	37	30	37	7
	3	11	—[5]	—[5]	6
	66	—[5]	49	71	85
	—[6]	—[6]	49	49	31
	—[6]	—[6]	48	56	46
	62	—[5]	72	76	65
Yeast B	91	88	98	87	79
	19	19	18	22	17
	158	—[5]	154	—[5]	156
	76	—[5]	75	—[5]	74
Yeast C	238	251	168	163	106
	152	149	104	101	69
	56	66	25	25	51
	53	38	26	27	56
	31	25	18	18	29

Missing data:

[5] Membranes ruptured
[6] Samples not run

The agreement in results obtained by the three methods is in a large part due to a larger than usual sample size (20 grams dse). Table 2 shows the agreement that would have been obtained if based on the individual results of the ABCB method

Table 2.—Yeast Counts Obtained by Plating Four Sets, Each Four Plates of 1.25 Grams dse.

	Average Calculated to 10 Grams dse	Individual Sets of 5 Grams dse Calculated to 10 Grams dse				Poisson Distribution 95% Assurance Based on 5 Grams dse Calculated to 10 Grams
		1	2	3	4	
Yeast A	108	94	108	110	120	80-138
	95	78	92	102	106	70-124
	41	34	34	38	58	26-60
	10	6	8	10	14	2-20
	25	12	18	28	40	12-40
	62	48	56	64	74	42-84
	7	2	4	12	10	0-16
	6	0	0	8	16	0-14
	85	76	82	84	96	62-112
	31	28	30	32	34	18-48
	46	36	42	46	60	28-66
	65	46	52	74	86	44-90
Yeast B	79	56	98	94	66	56-106
	17	22	18	18	10	6-30
	156	170	156	152	146	122-188
	74	70	84	72	66	52-98
Yeast C	106	90	128	122	84	78-136
	69	74	78	54	72	48-94
	51	54	46	50	52	34-72
	56	48	48	74	54	36-78
	29	30	24	42	20	16-46

using a sample size of five grams dse. The MF method permits the use of a sample size greater than 20 grams dse with resultant increased accuracy.

We have as yet found no yeast in liquid beet sugar products which shows a preference for high concentrations of sugar. It is, however, very likely that such a yeast may be present at times. It seems, therefore, necessary to test sugar products, in particular liquid sugars, for osmophilic as well as non-osmophilic yeasts. In order to arrive at a numerical evaluation it might be necessary to identify the yeasts as it could be expected that certain types, while inhibited somewhat by either medium, might develop under osmophilic as well as non-osmophilic conditions.

Three pure yeast cultures, supplied through the courtesy of Dr. Herman J. Phaff of the Department of Food Science and Technology, University of California at Davis, have very effectively shown the anticipated necessity of employing more than one medium.

These yeasts are:

Saccharomyces cerevisiae (low sugar tolerance)
Saccharomyces mellis (high sugar tolerance)
Saccharomyces rouxii (low and high sugar tolerance)

On tests run at the Spreckels Sugar Co. research laboratory it was found that the count of *S. cerevisiae* would be from three to five times greater on Mycophil broth than on osmophilic broth. *S. mellis* showed a definite preference for osmophilic broth and did not grow on Mycophil broth. *S. rouxii* grew vigorously on both media with possibly a slight preference for Mycophil broth.

The results obtained with the low and high sugar tolerant yeasts suggest the possibility that standards for yeasts may not have been devised on a completely rational basis. It seems likely that standards may have been set at unreasonably low values because of falsely low counts obtained when using a particular method. Even these lower standard values cannot give the desired protection if the yeast does not grow on the medium. Far better protection would appear possible, even with higher tolerances, if two types of media were used in the tests.

Summary

The use of molecular filter membranes for the detection and enumeration of yeasts in sugar products has not been completely accepted. This has principally been due to frequent lack of agreement with older established methods. It appears, however, that if due consideration is given to the growth requirements of the yeasts in regard to the selection of media, that equal or more accurate results may be obtained by the membrane method. Various yeasts found in sugar products may vary widely in growth requirements. It may, therefore, become necessary to differentiate between the yeasts which show optimum growth in low sugar solutions and those which prefer high sugar concentrations.

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Bulk and Liquid Sugar Production at the Brookfield (Chicago) Terminal

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The facilities at the Chicago Terminal were constructed to provide truck deliveries of the bulk dry and liquid sugars to customers in that area. The plant is located in the village of Brookfield about 12 miles southwest of downtown Chicago and is served by the C.B. & Q. Railroad.

The Terminal is capable of receiving annually over one million cwt of bulk sugar via railroad cars from the producing factories. It was designed to operate with a minimum of labor and at the same time produce liquid sugar of the very highest quality. The building was made attractive and the equipment was so arranged that the whole station could be kept clean with the minimum of effort.

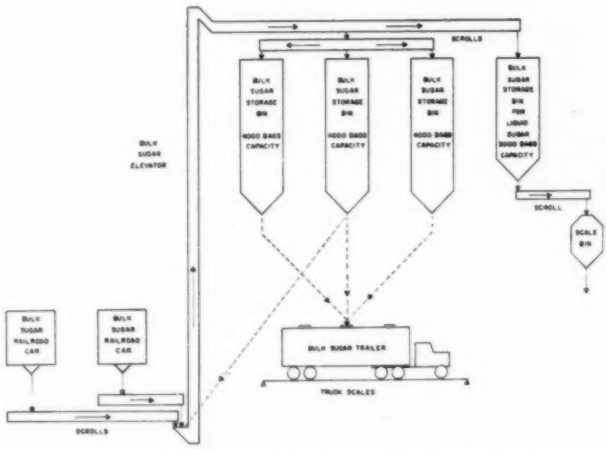

Figure 1.—Bulk sugar handling, Chicago Terminal.

A general arrangement of the bulk unloading and storage facilities is shown in Figure 1. Two parallel tracks under cover are provided with individual scrolls to the elevator. Provisions are made to unload either air-slide or hopper rail cars. Approximately three hours are required to unload an air-slide car.

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This sugar is conveyed to a bulk storage bin by means of conventional scrolls and bucket elevator. All sugar unloaded is passed through a magnet. A conveyor system over the bins is controlled remotely so that the sugar can be directed into any of the four 4000-bag bins. Three of the bins are used for sugar that is subsequently loaded as bulk granulated. The fourth bin is used for storage of sugar intended for the liquid station.

Bulk Loading

The loading of bulk trucks takes place inside and is accomplished simply by filling each compartment by gravity from the overhead bin while the truck is on the scale. The truck is moved back and forth to place the proper compartment under the bin for loading. Care must be taken to put the proper amount of sugar in each compartment so that individual axle loads are not exceeded. The sugar, when being loaded, is passed through a small mesh screen. The operation of loading a bulk truck is accomplished by one man with the aid of the truck driver. The complete operation takes approximately one and one-fourth hours. Both gravity and pneumatic type bulk trucks are used.

Liquid Loading

The other half of the loading station is devoted to liquid. A separate set of scales is provided. Liquid sugar, either sucrose at 67 Brix or invert at 76 Brix, is pumped to the station. The truck is loaded from the bottom through a special line which by-passes the trailer unloading pump. Remote controls are provided for the operator so that the tank trailers can be loaded by one man. He watches the scales and delivers the prescribed amount of liquid into the truck (approximately 3500 gallons), shuts the valve, which in turn automatically shuts down the pump. Separate pumps and lines are provided for invert and sucrose. The loading operation takes approximately 20 minutes.

Liquid Sugar Operations

The process for making liquid sugar is shown in Figure 2. The liquid sucrose process consists of dissolving sugar in demineralized water, treating the solution with carbon, filtering and cooling. When invert is being made the hot filtered solution is inverted with hydrochloric acid, neutralized with sodium carbonate and cooled. The plant is designed to produce either product but, since the same equipment is used, only one product is made at any one time. The process, except for the filtration station, is automated to minimize labor requirements.

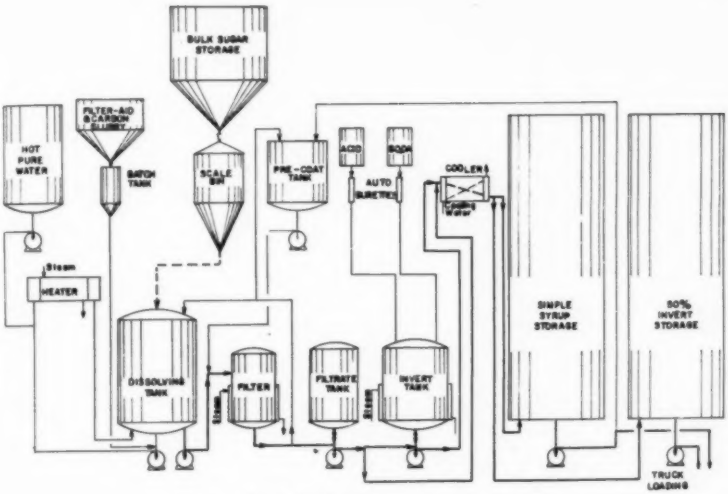

Figure 2.—Liquid sugar process flow.

The Dissolving Cycle

Sugar is weighed in batches of approximately 14,500 pounds and conveyed to the dissolving tank to which 900 gallons of water and carbon slurry have been added. As the sugar is introduced, the mixture is circulated through a stainless steel heater and maintained at 75° to 80° C. Two dissolving tanks are provided so that a continuous flow to the filter station can be maintained. The complete cycle is automated and controlled by a timer. All that is required of the operator is that he set the scale and meter for the prescribed amount of sugar and water and push a button to start each cycle. After completing the first cycle, he manually selects the other tank and starts the cycle again. A series of interlocks are provided in the electrical circuit to shut the operation down if any mechanical failure takes place.

After the dissolving cycle is completed the operator pushes a button that opens the drain valve and starts the filter pump. Three, 150 square feet horizontal plate, cartridge-type filters are provided, of which two are in service during operation. Suitable piping is provided so that the filters can be precoated and recirculated manually. After a filter is filled with cake, it is blown with air to remove the maximum amount of syrup and then put on circulation with water in the precoating system to extract as much sugar as possible from the cake. The cartridge is then removed, disassembled and redressed with paper. This

procedure of blowing, recycling and redressing requires approximately three hours. Two men are required during the cleaning of the cartridge. The syrup discharges from the filters into a stainless steel filtrate receiving tank. From this tank it is pumped through the heat exchanger to storage. A filtrate receiving tank is provided to minimize the back pressure on the filters.

A stainless steel, plate-type heat exchanger with 480 square feet of surface is provided to cool the syrup from 80° C. to 38° C. maximum. The syrup side of the cooler is multiple-pass with plates in parallel and series. Two sources of cooling water are used. First, the demineralized water is used to partially cool the syrup. Thus a portion of the heat is recovered. The remainder of the cooling is done with city water, which is subsequently sewered. A recirculating system, with temperature control of the cooling water, is provided on the final cooling so that at no time can the water cool the syrup to the point that pressure-drop across the cooler becomes excessive. This becomes important when cooling 76 Brix invert syrup.

The liquid sugar is discharged directly from the cooler into storage tanks. The pressure on the cooler varies with the head in the storage tank. Two steel storage tanks of 15,000 gallons each are provided for liquid sucrose. These tanks are 10½ feet in diameter and 24 feet high with a dished head on top and a flat sloping bottom. All corners are rounded and ground flush. The inside surface is coated with a resin paint. Ultra-violet lights are provided in the roof. A double-filtered air circulating system continuously circulates air. Each tank is provided with a side-entering mixer having mechanical seals with water lubrication. A washing nozzle approximately six feet from the bottom is provided to accept a power rotating washing spray. The outlet nozzle is located twelve inches off the bottom with an auxiliary nozzle on the bottom for complete drainage. Each tank has five nozzles below the liquid level. Every effort was made to minimize places that may create contamination of the product and render washing ineffective. Blank covers are provided to replace the ultra-violet lights during washing. Pressure diaphragms are placed in the bottom discharge line so that tank levels can be recorded on the instrument panel.

The Invert Cycle

The previous description of the dissolving cycle also applies when making invert. The ratio of sugar and water is changed so that approximately 73 Brix simple syrup is produced. The cycle timing is changed slightly to allow for a somewhat longer dissolving period. The volume in the carbon measuring tank is

increased to allow for the greater quantity of sugar in each batch. The operator then proceeds as described previously. Flows through the filter station to the filtrate receiving tank are reduced to allow for the increased viscosity of the higher Brix syrup. The operator must now start the cycle that directs the syrup to the inversion tanks. There are four glass-lined, jacketed, 1000 gallon inversion tanks, each controlled by a sequence timer. This timer performs each step in the operation—that is, opens the fill valve, starts the pump, stops the pump at the proper volume, closes the valve, brings the tank to the prescribed temperature, shuts off the steam, adds the acid, allows the proper time for inversion, adds the sodium carbonate to stop inversion, opens the drain valve, and starts the discharge pump to pump the invert through the cooler to storage. Burettes are used for measuring the acid and sodium carbonate solutions. The timer circuit also includes filling these burettes and checking to determine that they are full before discharging and empty before refilling—precautions, to assure that the proper amounts of hydrochloric acid and sodium carbonate are delivered to each batch of invert.

One master timer controls the over-all operation; each of the four inversion tanks have timers. Timers start the cycle of each tank in series. After the master timer starts the cycle for a tank, the individual timer takes over and finishes the inversion cycle. The individual timer delays the draining of the tank until the previous tank has been drained and pumped through the cooler. After the tank is drained, the individual timer resets itself and delays the next cycle until the master timer has started the cycle for each inversion tank. During the period of making invert the operator is primarily concerned with operating the dissolving cycle to supply syrup to the inversion station. The inversion conditions used to produce 55 percent invert at 76 Brix are approximately 2.3 pH, 80 to 83° C. and 40 minutes. Slight variations occur which are usually compensated for by adjusting the time of inversion.

The syrup leaving the station is pumped through the cooler to storage. Since the operation of the cooler is in batches of 1000 gallons and inevitably a short delay occurs between each batch, the matter of temperature control of this cooler becomes somewhat more elaborate than might be thought necessary. As described before, two water supplies are used for the sake of heat and water economy. The demineralized water used in the dissolving cycle is the cooling medium in the first half of the cooler. This water leaves the cooler at approximately 60° C.

The amount of pure water used is regulated manually so that a supply of hot, pure water is available for dissolving the sugar. The balance of the cooling is then accomplished with city water. The automatic control of the cooler is accomplished by regulating the amount of recycled city water bled into the system. A three-way valve controls this quantity of hot water bled into the recirculating system, thus maintaining a predetermined temperature of the cooling water going to the cooler. Thus, two temperature controls are used, (1) to control the temperature of the final cooling water, and (2) to control the temperature of the syrup and, by consequence of the increased pressure drop, limiting the flow through the cooler. The problem of overcooling the syrup is, of course, magnified by the intermittent operation associated with automated batch operation.

Two 15,000 gallon storage tanks are provided for invert syrup, the same as for liquid sucrose. Since the same cooler is used for both operations some precaution is necessary to avoid contamination of simple syrup with invert when switching operations. This is simply accomplished by pumping a small quantity of simple syrup through the system to the invert storage tanks before switching the pipe lines to the simple syrup storage.

Auxiliary Equipment

Boiler

A 100 horsepower boiler, generating 3500 pounds of steam per hour, designed for completely automatic operation, has been installed. This gas- or oil-fired boiler provides the process and heating steam necessary.

Demineralizer

A two-bed water demineralizer is provided to produce .5 grain water. This unit has a flow rate of ten gallons per minute and total capacity of 42,000 gallons. The unit is manually operated and regenerated. The only instrumentation required is a conductivity cell that shuts the unit off at a predetermined, maximum allowable conductivity. A float valve is provided in the demineralized water supply tank so that the unit can be operated at night with no attendant. This assures a full supply of demineralized water each morning. A booster pump is provided on the city water supply to increase the pressure, if needed.

Carbon Slurry Equipment

A resin-coated agitated tank is provided for carbon filter aid slurry. Carbon, filter aid and water are added to this tank to make a twenty percent slurry. This station is in a room that is completely isolated from the rest of the plant. A ventilating

system is provided for this tank. Every precaution has been taken to minimize carbon from being carried to other parts of the plant.

The carbon is introduced into the process as a part of the automatic dissolving cycle. A flush bottom valve opens on the slurry tank which fills a small measuring tank by gravity. The float valve on the measuring tank closes this valve and opens the valve on the measuring tank allowing the carbon slurry to be fed into the process. This slurry is introduced into the suction side of the pump circulating syrup through the heater. By adjusting the level in the measuring tank the carbon dosage can be changed as desired.

Precoating Equipment

An agitated, resin-lined tank and stainless steel pump are provided for precoating operations. Simple syrup is used either from the process or storage to slurry the precoat material. A mixture of filter aid and asbestos is added in the amount of 20 pounds per 100 square feet of filter surface. This slurry is then pumped through the filter back to the precoating tank until the material has been deposited on the paper filter medium. This system is also used to remove the sugar from the carbon cake at the end of a filter cycle. Water is recirculated in this system through the filter to reduce the sugar content of the carbon cake. The sweet water is then disposed of by addition to the next dissolving cycle.

Tank Washing Equipment

A two-tank washing system is provided to wash and sterilize tank trailers and any equipment in the plant. This system consists of two resin-lined tanks, a heater, a high pressure pump, a portable, rotating nozzle and return pump. The usual cleaning operation on a tank trailer consists of recirculating the cleaning solution through the spray nozzles for a period long enough for the nozzle to complete three cycles. Subsequently, two washes of hot water are used. The nozzle is allowed one complete cycle for each rinse.

Quality Control of Liquid Sugar

Every step to insure the quality of the liquid sugar has been taken. Superb filtration is accomplished by the use of horizontal plate filters. The turbidity of the liquid sugar produced is beyond reproach. Stainless steel is used throughout to insure the minimum of product contamination. Adequate carbon dosage is used to insure an extremely low solution color and absence of floc. The conductivity is kept low by the use of demineral-

ized water. Every precaution for maintaining sanitary conditions has been provided. The tank trucks and storage tanks are sanitized on a schedule. Bacteriological control is conducted by a commercial laboratory in the area. The following is a typical example of the liquid products produced.

	Liquid Sucrose	Liquid Invert
Solids (RDS)	67.0	75.0
Invert on solids	0.0	55.0
Sucrose on solids	67.0	45.0
pH	5.8	4.6
Reference Basis Color	3.0	11.0
Turbidity	0.3	0.3
Floc	1.0	1.0
Specific Conductivity $\times 10^{-5}$	2.2	7.0
Sediment (white disc)	0	0
Sediment (black disc)	0	0
Odor	0	0
Bacteria		
Flat Sour	0	0
Total thermophiles	0	0
Mesophiles per 10 g sugar	10	10
Yeast and mold/10 g DS	0-2	0-2

At present the process is operated eight hours per day. No difficulties of any kind have developed to mar the quality of the product. Possibly the reason intermittent operation does not produce high bacteria counts is that all filters and pipe lines in process are kept full at all times. With the periodic cleaning of tank trailers and storage equipment no serious bacteria contamination has been encountered.

An Investigation of the Effects of Various Methods of Herbicide Application on Weed Control in Sugar Beets

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At the present time, there is considerable controversy about the value of incorporating herbicides with the soil. Although many herbicides may not need to be incorporated, soil incorporation can reduce loss as vapors, lessen risk of crop injury, and improve weed control (5)[2]. Poor weed control has been associated with wet soil conditions at the time of pre-emergence application of EPTC (ethyl N, N- di-n-propylthiolcarbamate) (1). However, investigations have indicated that this chemical gives reliable weed control when incorporated with the soil. The effects of both incorporation and irrigation on the activity of EPTC, CDEC and CIPC have been investigated by Havis et al. (4). Endothal (3,6-endoxohexahydrophthallic acid) also has been reported as being more effective when incorporated (3).

The tests reported below were designed to evaluate the effect of two chemicals; endothal and EPTC, on weeds and sugar beets, under different methods of application.

EPTC at 2,4 and 6 and endothal at 4,6 and 8 pounds of active material per acre were applied in a split-plot design consisting of rates of chemicals and methods of applications on 9 x 20 foot plots replicated six times. Chemicals were applied in 30 gallons of water per acre by an Oxford Precision sprayer to a freshly worked, packed and moist soil surface. There were four methods of applications: (1) pre-emergence (after beets planted) surface application, (2) incorporation (before beets planted) with a spike-tooth harrow, (3) incorporation with a spring-tooth cultivator, (4) incorporation with a double disk. All plots were then harrowed and packed. All incorporation plots were sprayed early May 9 and incorporated immediately. Monogerm sugar beets[3] were planted May 11, 1959 and the pre-emergence plots treated the same day.

Effects on beets and weeds were estimated and counts were made on June 8 and 9, 1959. Weeds present were mainly redroot pigweed, (*Amaranthus retroflexus* L.), lamb's quarters

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[1] Departments of Field Husbandry and Botany, Ontario Agricultural College, Guelph, Canada.

[2] Numbers in parentheses refer to literature cited.

[3] Monogerm seed was a blend of U158-K114 and SL227MSxSP5114-0 and SL117MSx-SP5481-0.

Table 1.—Effects of Chemicals and Methods of Application on Weeds and Beets.

Treatment	Pre-Emergence Surface					Spike-Tooth Harrow				
	Weed Count		Herbicidal Rating		Beet Injury	Weed Count		Herbicidal Rating		Beet Injury
	Grass	BLW[2]	Grass	BLW	% of Check	Grass	BLW	Grass	BLW	% of Check
EPTC 2 lb./A.[1]	5.8	19	221	92	0	1.8	10	896	517	6
EPTC 4 lb./A.	7.8	19	238	127	0	1.7	7	936	849	26
EPTC 6 lb./A.	5.7	14	288	240	0	1.0	4	957	939	36
EPTC Mean	6.4	17	249	153		1.5	7	930	768	
Endothal 4 lb./A.	5.4	16	286	116	0	4.8	12	512	382	0
Endothal 6 lb./A.	5.7	15	214	285	0	4.0	10	612	438	0
Endothal 8 lb./A.	4.2	16	251	270	0	2.4	11	622	378	0
Endothal Mean	5.1	16	250	224		3.7	11	582	400	
Check	6.9	18	0	0	0	10.7	19	0	0	0

Treatment	Spring-Tooth Cultivator					Double Disk					Mean			
	Weed Count		Herbicidal Rating		Beet Injury	Weed Count		Herbicidal Rating		Beet Injury	Weed Count		Herbicidal Rating	
	Grass	BLW	Grass	BLW	% of Check	Grass	BLW	Grass	BLW	% of Check	Grass	BLW	Grass	BLW
EPTC 2 lb./A.	1.5	7	755	828	12	.2	4	925	861	6	2.3	10	699	574
EPTC 4 lb./A.	0.	4	972	933	26	.5	3	929	975	26	2.5	8	769	721
EPTC 6 lb./A.	.2	3	973	985	34	.2	2	978	982	56	1.8	6	799	787
EPTC Mean	.5	4	900	915		.3	3	944	939		2.2	8	756	694
Endothal 4 lb./A.	3.3	14	312	158	0	1.0	11	566	365	0	3.6	13	419	255
Endothal 6 lb./A.	6.7	14	439	254	0	2.4	15	399	266	0	4.7	14	416	311
Endothal 8 lb./A.	5.7	14	289	202	0	3.0	11	245	438	0	3.8	13	351	322
Endothal Mean	5.2	14	347	204		2.1	12	403	356		4.8	13	396	296
Check	2.6	14	0	0	0	2.8	27	0	0	0	5.8	20	0	0

[1] All rates are in terms of active material per acre.

[2] BLW = broadleaf weeds.

[3] Herbicidal rating = $1000 - \left[\frac{\text{Weed count (\% of check)} \times \text{Weed injury (\% of check)}}{10}\right]$

(*Chenopodium album* L.) and barnyard grass (*Echinochloa crusgalli* L. Beauv.). An analysis of variance was performed on grass count, broadleaf weed count and herbicidal ratings in which the main effects and interaction were broken down into components, each having one degree of freedom. Results are presented in Table 1.

The data in Table 1 show that the grass control obtained with endothal tended to be increased where this chemical was incorporated into the soil with a spike-tooth harrow. However, this method of incorporation was not significantly better than the other methods of application when compared by the analysis of variance technique. Furthermore, the data show that there was no organized trend of effectiveness in either broadleaf weed or grass control when endothal levels were increased.

There was no difference in grass control between different levels of EPTC but broadleaf weed control was significantly increased as the level of EPTC was increased. These trends were observed under all methods of application.

The weed control effectiveness of EPTC on both grasses and broadleaf weeds increased markedly when it was incorporated with the soil rather than applied to the soil surface. However, incorporation by the use of a double disc or a spring-tooth cultivator gave more effective broadleaf weed control than incorporation by means of a spike-tooth harrow. No such difference was noted with respect to grasses.

The treatment that gave the best general weed control with the least beet injury appeared to be EPTC at two pounds per acre, incorporated either by double disking or spring-tooth cultivation immediately after application.

Summary

EPTC at 2, 4 and 6 and endothal at 4, 6 and 8 pounds active material per acre were applied in a split-plot design consisting of rates of chemicals and methods of application on 9 x 20 foot plots replicated six times. Methods consisted of: (1) surface application, (2) incorporation with a spike-tooth harrow, (3) incorporation with a spring-tooth cultivator and (4) incorporation with a double disk. At the time of application, the soil was moist.

Endothal gave unsatisfactory reduction of weeds and grasses under all methods of application and at all rates. Endothal when incorporated with a spike-tooth harrow gave greater grass reduction. All other methods of application of endothal gave similar results with both broadleaf weeds and grasses.

EPTC gave no weed reduction and no beet injury when applied pre-emergence to the soil surface but was considerably better when incorporated. Incorporation with the double disk and the spring-tooth cultivator was significantly better than with the spike-tooth harrow in the control of broadleaf weeds but not in the control of grasses. As the rate of EPTC increased broadleaf weed control increased but beet stunting was severe at the highest rate. The treatment giving the best general weed control with least beet injury appeared to be EPTC at two pounds per acre, incorporated either by double disking or spring-tooth cultivation immediately after application.

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Selection of Sugar Beets for Tolerance to Endothal Herbicide

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Some injury to sugar beets has been observed in the seedling stage where the herbicide endothal (disodium 3, 6-endoxohexahydrophthalate) was used at rates sufficiently high to give good weed control. Comparative observations in commercial sugar beet fields have frequently shown as much as 15% reduction in prethinning stand on endothal treated areas. Also, over-all seedling vigor of surviving plants is generally retarded but occasional beets have shown no apparent injury from the endothal used for weed control. This suggested a genetic difference in tolerance to endothal between beets of a variety; and on this assumption, selection for tolerance was initiated in December of 1957.

First attempts at finding endothal tolerant sugar beets included the germination of seed on blotters, and on agar, impregnated with a solution of endothal. Before any beets were actually saved for variety improvement and progeny tests these methods were replaced by growing beets in coarse sand moistened with endothal solution.

The first selection was made on a monogerm variety. Selection by two different criteria was done in the greenhouse using flats three inches in depth as containers. Selection A was made on the basis of normal development of primary root on seedlings growing in flats treated at a rate of 10 pounds of endothal per acre. Selection B was based on seedlings surviving a rate of 140 pounds of endothal per acre. In both cases, the endothal was applied at time of planting and was mixed in sufficient water so that the entire volume of sand was moistened.

Approximately 120 plants were selected for each group and the selected plants amounted to about 4% of the parent population. The selected plants were grown in the greenhouse for rapid seed production, and immediately on getting the seed, a progeny test was run using flats and coarse sand. Rates of endothal of 10 and 140 pounds per acre were again used. The results of growing seedlings of the original parent seed and seed of Selections A and B at the 10-pound rate of endothal showed no difference in stand, but more vigor was noted in seedlings of the selections. The progeny test made at the 140-pound rate was

[1] Agronomist, Agronomist, and Plant Breeder, respectively, Agricultural Experiment Station, The Great Western Sugar Company, Longmont, Colorado.

in favor of the selections both in vigor and stand in spite of their apparent poorer seed quality as compared with the parent seed. Percentage emergence and percentage survival of seedlings at the 140-pound rate were equal. Based on monolocular seed units planted, percentage survival within each of the three groups was as follows:

Parent	9.5% survival
Selection A	13.5% survival
Selection B	21.5% survival

This first progeny test was completed during the period September 4 to October 4, 1958. The survival of plants of the parent was about twice as high as was obtained in December of 1957. This is assumed to be environmental effect of temperature and moisture and possibly light which were not kept constant. This does not distract from the gain in survival of the selections over the parent which from greenhouse results was taken to be substantial, and particularly in case of the Selection B.

Further testing of the parent and progeny was conducted in a replicated test in the field during 1959. A randomized split plot design was used. The variables being the three beet varieties and four endothal rates, namely 0, 1½, 3, and 4½ pounds per acre applied as a band treatment just ahead of planting. Usual commercial use has been ¾ to 1½ pounds per acre. The equivalent broadcast rates would be 3⅔ times these rates.

Endothal application and planting was completed April 29. There was a total of eight replications, four of which were sprinkle irrigated immediately after planting. Sprinkling had no apparent effect as ample rain was received. Data of eight replications were therefore combined. Pre-thinning counts of beets and weeds were completed on June 4. Thinning and removal of all weeds was completed by manual means June 9. Photographs of seedling beets were taken as record of seedling vigor.

Stand and weight of beets at harvest, October 22, were based on six rows per plot, each row being 18 feet in length. Sugar percentage and thin juice apparent purity were based on beets from only two rows per plot. Data obtained appear in Tables 1, 2, and 3.

In Table 3 the harvest results show no difference for any of the measurements whether analyzed to measure differences between varieties or rates of endothal application. From the results of Table 3, and from other analysis of the data which has been made but not reported here, one finds no interaction of variety

and rate of endothal. Since the maximum rate of endothal used did not depress yield or quality of any of the varieties, one has to conclude the rates of endothal were too low to differentiate varieties. Table 1 shows no major difference in emergence or stand of beets which can be attributed to endothal rate. The difference between results of varieties presented in Table 1 can more logically be attributed to difference in seeding rate. The seed of Selection A and Selection B was smaller and more seed units were planted in comparison with the parent variety.

Table 1.—Summary of Pre-Thinning Beet Stands.

Rate of Endothal	Variety		
	Parent	Selection A	Selection B
(Pounds per acre)		(Beets per 100 inches)	
0	96[1]	133[1]	128[1]
1½[2]	107	141	146
3	103	120	127
4½	99	113	115

[1] Within a variety the differences from check are not significant at 5% point as calculated from 4-50 inch counts per plot.

[2] Broadcast rate would be 3⅜ times indicated amount.

Table 2.—Average Weed Count per Treatment and Percentage Kill of Weeds Obtained with Each Endothal Rate.

Rate of Endothal	Plots of			Weed Kill
	Parent	Selection A	Selection B	
(Pounds per acre)	(Weeds per 40 feet of row)			(Percent)
0	41.4[1]	37.7	34.1	0
1½	6.2	9.5	8.2	78.9[2]
3	3.6	4.1	1.6	91.8
4½	3.7	2.1	1.4	93.6

[1] Weeds remaining in row after first cultivation.

[2] Difference in weed control between 1½, 3, and 4½ pound rates is not significant at 5% point based on counts of 40 feet of row per plot. Difference between any endothal treatment and zero treatment is highly significant.

Table 3.—Harvest Results Averaged for Variety and Endothal Rate, Respectively, Longmont, Colorado—1959.

	Beets per 100 ft. (No.)	Beets per Acre (Tons)	Sugar Content (%)	Thin Juice Apparent Purity[1] (%)	Gross Sugar per Acre (Lbs.)
Variety results					
Parent	122	22.2	17.80	95.0	7907
Selection A	122	22.3	17.86	95.4	7955
Selection B	120	22.0	17.91	95.1	7880
LSD 5% pt.	.....	1.1	0.45	0.76	435
Endothal rate results					
No Endothal	120	22.2	17.96	95.3	7989
1.5 lb. per acre	120	22.2	17.89	95.3	7943
3.0 lb. per acre	122	22.3	17.81	95.0	7950
4.5 lb. per acre	124	21.9	17.77	94.9	7780
LSD 5% pt.	.....	1.0	0.41	0.70	392

[1] Analysis run on press juice.

Although the pre-thinning stand and harvest data from the field trial show no difference between varieties, the vigor of beets prior to thininng was quite different. One could observe visually that at the higher endothal rates the seedling vigor of the parent variety was very low, that of Selection A was intermediate, and Selection B was most vigorous, showing little retardation in growth. The weed control obtained and reported in Table 2 leaves no doubt but that the endothal was in the soil even where Selection B showed negligible stunting.

Further selection has been made within Selection B in an effort to further increase endothal tolerance. The overall results with selection B have been sufficiently favorable to prompt commercial seed increase of this variety. Further experience will dictate whether all varieties produced will go through endothal tolerance selection, or at least an endothal tolerance rating, before being released to growers in Great Western and Northern Ohio Sugar Company operating territories, where endothal herbicide is presently being used on a large percentage of the acreage planted to sugar beets.

Summary

Selection within a monolocular sugar beet variety was made for increased tolerance to the herbicide endothal. Results of progeny tests indicate that endothal tolerance was improved by selection. The evaluation of improvement is based on emergence and survival of beet seedlings grown in the greenhouse under very high endothal rates. Selected progeny tested under moderately high endothal treatment in a field trial showed an increase in seedling vigor over the parent. Harvest data did not prove superiority of selected progeny over the parent, but showed them to be equal. The endothal rates used in the field trial presumably were too low to give definitive results at harvest time. Work on further improving varieties for endothal tolerance is in progress since presently it is considered feasible and of value.

Further Studies on the Control of Weeds in Sugar Beets with Herbicides

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Promising herbicides from last year's experiments, in addition to several new herbicides, were tested on sugar beets during 1959 in preplanting soil incorporated and pre-emergence soil surface experiments at the Ontario Agricultural College (1)[2]. Both experiments included the same treatments in a 6 x 6 simple lattice design with two replications allowing for 36 treatments. Two check plots were included in each replication. Plot size was 9 x 20 feet, each plot containing four rows of sugar beets.

The pre-emergence plots were planted May 11, 1959, and treatments for both experiments were applied to the soil surface on May 12. In the preplant experiment, incorporation of the herbicides into the soil was carried out by means of a double disk, followed by a spike-tooth harrow and packer, on the evening of May 12 with sugar beets being planted the following morning. Both experiments were conducted on Burford loam soil with monogerm sugar beet seed[3] being used.

All liquid herbicides were mixed with water and applied with an Oxford precision sprayer at the rate of 30 gallons per acre. The granular herbicides were applied with a Gandy seeder. At the time of treatment the soil was moist and continued moist for some time. Weed counts and ratings were made on June 9 and 10. No yields were taken.

Weeds present were mainly lamb's-quarters (*Chenopodium album* L.) and redroot pigweed (*Amaranthus retroflexus* L.) with few annual grasses.

The data presented in Tables 1 and 2 show that the liquid formulation of EPTC had little effect on broadleaf weeds. This was to be expected in the case of the pre-emergence surface application but not in the incorporation trial. Since there was an eight hour delay between surface application and incorporation, the chemical may have escaped by volatilization from the moist soil surface. Granular EPTC gave poor weed control when applied pre-emergence to the soil surface but excellent weed

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[2] Numbers in parentheses refer to literature cited.

[3] Monogerm seed was a blend of UI58-K114 and SL117MSxSP5714-0 and SL117MSx-SP5481-0.

Table 1.—Effect of Pre-Emergence Herbicides on Weeds and Sugar Beets.

Treatment	Rate[1]	Weeds/Sq. Ft.[2]		Injury Ratings % of Check		Herbicidal[3] Rating
		Mean	% of Check	Beets	Weeds	
EPTC liquid	2	8.00	96.2	100	100	38
	4	10.36	100.	100	100	0
	6	10.29	100.	100	100	0
EPTC granular	2	9.82	100.	100	100	0
	4	6.02	72.4	100	100	276
	6	3.33	40.	95	90	640
Monuron	½	1.07	12.9	83	55	929
	¾	.66	7.9	63	40	968
	1	0	0	55		1000
Diuron	½	.72	8.6	63	47	960
	¾	1.10	13.2	38	5	993
	1	0	0	5		1000
Endothal	4	5.25	63.1	100	100	369
	6	5.15	61.9	100	100	381
	8	2.57	30.9	100	87	731
Endothal Oil Susp.	4	2.76	33.2	100	100	668
	6	6.40	76.9	100	100	231
Endothal granular	4	5.65	67.9	100	100	321
	6	3.84	46.2	100	77	644
	8	4.20	50.5	96	73	631
GC 2603[4]	1	1.05	12.6	83	45	943
	2	0	0	22		1000
	3	.25	3.0	25	5	999
CP 6936[5]	4	2.39	28.7	100	73	790
	6	3.00	36.	100	67	759
	8	1.28	15.4	93	35	946
Niagara 5996	½	7.12	85.6	100	100	144
	1	5.25	63.1	78	100	369
	1½	2.42	29.1	35	83	758
CDEC	4	4.76	57.2	100	67	617
	6	1.92	23.1	100	53	878
	8	3.16	37.9	100	43	837
CIPC + CDEC	2,4	1.59	19.1	83	67	872
	3,4	.17	2.0	45	45	991
Check		8.32	100.	100	100	0

[1] All rates are pounds of active material per acre.

[2] Average of 8, one square foot samples (4 per plot in each replicate).

[3] Herbicidal rating $= 1000 - \left[\frac{\text{Weed injury (\% of check)} \times \text{weed control (\% of check)}}{10}\right]$

[4] GC 2603 = 3-phenyl-1, 1-dimethylurea trichloroacetate.

[5] CP 6936 = alpha-chloro-N-ethyl-N-phenylcarbamate.

Table 2.—Effect of Pre-Planting Incorporated Herbicides on Weeds and Sugar Beets.

Treatment	Rate[1]	Weeds/Sq. Ft.[2]		Injury Ratings % of Check		Herbicidal[3] Rating
		Mean	% of Check	Beets	Weeds	
EPTC liquid	2	7.35	82.2	100	100	178
	4	8.67	97.	100	100	30
	6	4.90	54.8	87	97	468
EPTC granular	2	1.43	16.	73	73	883
	4	.28	3.1	40	13	996
	6	.19	2.1	14	13	997
Monuron	½	1.86	20.81	56	63	869
	¾	.64	7.2	43	45	968
	1	.08	.9	44	45	996
Diuron	½	4.81	53.8	100	75	596
	¾	2.09	23.4	63	63	853
	1	1.69	18.9	59	43	919
Endothal	4	4.66	52.1	100	100	479
	6	1.37	15.3	100	55	916
	8	6.29	70.4	100	83	416
Endothal Oil Susp.	4	8.67	97.	100	100	30
	6	8.73	97.7	100	100	23
Endothal granular	4	3.33	37.3	100	70	739
	6	3.49	39.1	94	67	738
	8	1.86	20.8	97	57	881
GC 2603[4]	1	2.61	29.2	66	53	845
	2	.61	6.8	46	27	982
	3	.85	9.5	13	15	986
CP 6936[5]	4	6.08	68.	100	93	368
	6	5.10	57.1	100	93	469
	8	2.24	25.1	100	75	812
Niagara 5996	½	6.67	74.6	100	100	254
	1	6.13	68.6	81	97	335
	1½	3.12	34.9	28	37	871
CDEC	4	4.15	46.4	100	95	559
	6	5.70	63.8	100	70	553
	8	6.40	71.6	100	95	320
CIPC + CDEC	2,4	3.75	41.9	83	77	677
	3,4	1.66	18.6	65	40	879
Check		8.94	100.	100	0	

[1] All rates are pounds of active material per acre.
[2] Average of 8, one square foot samples (4 per plot in each replicate).
[3] Herbicidal rating $= 1000 - \left[\frac{\text{Weed injury (\% of check)} \times \text{weed control (\% of check)}}{10}\right]$
[4] GC 2603 = 3-phenyl-1, 1-dimethylurea trichloroacetate.
[5] CP 6936 = alpha-chloro-N-ethyl-N-phenylcarbamate.

control was obtained even at the two-pound rate when it was incorporated before planting. This would indicate a greater stability in the granular formulation than in the liquid.

Monuron and Diuron gave excellent weed control at all rates. Both were slightly better when applied pre-emergence to the soil surface than when incorporated. However, both injured the beets considerably and would appear to be too toxic to be used in this crop.

Both the water soluble and emulsifiable formulations of endothal gave erractic weed control. Granular endothal seemed to give better weed control, especially when incorporated, with slight beet stunting. GC 2603 gave excellent weed control in both trials with fairly severe beet injury at the two-and three-pounds rate. CP 6936 gave consistently good results under the pre-emergence application with no beet injury, but seemed to cause more beet injury with less weed control when incorporated. Niagara 5996 gave satisfactory weed control but severe beet injury at the rate of 1.5 pounds per acre. CDEC gave satisfactory weed control at six pounds per acre applied pre-emergence to the soil surface. There was no beet injury caused by this chemical in any treatment. A mixture of CIPC at two pounds and CDEC at four pounds per acre applied as a pre-emergence surface application gave satisfactory weed control with slight beet injury.

Summary

Several herbicides that had been shown to be promising for the control of weeds in sugar beets, and two new materials, were tested in preplanting soil incorporated and pre-emergence soil surface applications during 1959. Granular EPTC at 2 pounds per acre was the most satisfactory preplanting soil incorporated treatment. Granular endothal with similar application was also good. CDEC and CP 6936 were the best pre-emergence soil surface treatments as they gave good weed control and no beet injury. Liquid EPTC and both the water soluble and emulsifiable forms of endothal were not satisfactory under either method of application. Monuron, Diuron and GC 2603 gave good weed control but damaged the crop extensively.

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Some Effects of Virus Yellows on Sugar Beet Processing Quality

A. E. Goodban, A. I. Morgan, R. Teranishi, H. G. Walker, Jr., and R. M. McCready[1]

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In recent years the disease, virus yellows, has become of increasing importance to the U. S. sugar beet industry, especially in some far western sections. Considerable evidence has been accumulated, both in Europe and the United States, that infection by the yellows virus can markedly reduce yield, sucrose content, and purity of beets. Bennett, Price, and McFarlane (1) have recently reported that factors such as fertilization level, beet variety, strain of virus, and maturity of beets at the time of infection are important in determining the extent of damage to the crop. Very little is known, on the other hand, of the effects of virus yellows infection on the processing quality of beets. The work reported here was undertaken to provide some answers to the question of processing quality effects such as sedimentation and filtration rates, lime salts, color, purity, and nitrogen levels.

In the fall of 1957 we obtained from J. S. McFarlane some beets which were grown at Salinas, California, as part of the yellows resistance breeding program of the U. S. Department of Agriculture. The variety was 5554H1, which is an F_1 hybrid between two inbred lines. The beets were planted December 16, 1956, and inoculated with virus yellows on April 15, 1957. A control planting of the same variety was sprayed regularly to control the aphid vector. Spraying greatly delayed infection of these beets, but by harvest time (August 20) almost all of the beets showed symptoms of yellows. Table 1 shows data supplied by Dr. McFarlane on the field effects of the virus infection. Considering the high yield of 34 tons per acre, it appears that virus effects in the control beets were slight and that the two batches of beets give a fair comparison between healthy and infected beets. The differences in yield are highly significant.

The beets were processed in the small-scale processing laboratory at Albany, California, (2) after 45 days' storage at 1° C. The Olsen diffuser was operated at 70° C., draft 135, and a retention time of 45 minutes, with cossettes having an average

[1] Western Regional Research Laboratory, Albany, California, a laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

length of 14 to 18 meters per 100 g. The diffusion juice was limed and carbonated immediately in the model Dorr carbonator (3) at 80° C., using 2% lime on beets, 23 minutes retention time, and a recycle ratio of 7 to 1. Final first carbonation alkalinity was .078 and .111% CaO for the control and .089 and .110% CaO for the infected beets. The two lower

Table 1.—Effect of Virus Yellows on Beet Growth.

	Control	Inoculated	Loss %
Yield, tons/acre	34.09	21.58	36.8
Gross sugar, tons/acre	5.48	3.21	41.4
Sucrose, % on beet	16.08	14.90	7.3

Table 2.—Effect of Virus Yellows on Diffusion.

	Control	Inoculated
Beets:		
Polarization, %	18.01	16.73
Apparent purity, %	87.2	86.2
Total N, %	0.17	0.19
Pulp:		
Total N, %	0.09	0.09
Apparent purity, %	81.3	71.2
Diffusion juice:		
Apparent purity, %	93.2	92.3

Table 3.—Effect of Virus Yellows on Carbonation.

	Control	Inoculated	
1st carb.:			
Sedimentation rate	17	14	lbs./ft.^{2} hr.
Filtration rate	107	32	
2nd carb.:			
Total N	435	560	mg./liter, 10% RDS
Color	.12	.15	O.D., 10% RDS[1]
Lime salts	.012	.014	g. CaO/100 Brix
Purity	95.4%	95.2%	pol/RDS × 100

[1] Adjusted to pH 7, optical density at 420 less optical density at 720 mμ, in Spectronic 20 spectrophotometer.

alkalinities corresponded to pH 10.15, measured at 80° C. The processing characteristics of the two samples are shown in Tables 2 and 3. Table 2 compares the two samples for the diffusion step.

Beet polarization at time of processing was higher than at harvest time, indicating that both batches dehydrated approximately 10% during storage, but the ratio of sugar contents between control and inoculated beets is the same as at harvest time. There was no spoilage apparent in the beets when they were taken out of storage. Pulp and beet purities were calculated from the equation

$$P = \frac{S}{R} \times \frac{100\text{-}R}{100\text{-}T} \times 100$$

where P = purity, %
S = beet polarization, %
R = press juice refractometric dry solids (RDS), %
T = beet dry solids, %

This purity is not comparable to the diffusion juice purity but it is a measure of the difference between the control and infected beets. The infected beets show the same non-sugar elimination during diffusion as the control, as evidenced by pulp and diffusion juice purity, even though extraction was more complete for the control beets. The infected beets show a higher total nitrogen content, and, furthermore, this increase is carried through to the diffusion juice, since the same amount of nitrogen is retained in the pulp in each case.

Equilibrium was considered to be established when conditions on the carbonation station had been constant for one hour, which is approximately 3 retention times. At this time 95% of the material in the system has been replaced. The sedimentation and filtration rates were then measured, and a sample of first carbonation juice filtrate was subjected to batch second carbonation. This was accomplished by heating the filtrate to a boil in a steam-jacketed pot, gassing for 3 minutes with CO_2 followed by boiling for 5 minutes to remove excess CO_2. The juice was filtered immediately and analyzed for sucrose, RDS, total nitrogen, color, and lime salts. Results are shown in Table 3. All data in the table are averaged from runs at pH 10.15 and at alkalinity 0.11% CaO for each sample of beets.

The most noticeable effects of yellows damage are in filtration rate, total N, and sedimentation rate. The color difference is significant but is difficult to interpret, since no information

is available on how much of the thin juice color is retained by the white sugar. The lime salts and purity differences between healthy and infected beets are not statistically significant.

In this test yellows-affected beets gave only slightly poorer thin juice, but filtration was markedly lower, as compared with the control beets. Infected beets produced more soluble nitrogen than the controls. Although changes in processing quality as a result of yellows infection were rather minor, they were all toward poorer-quality juices.

Acknowledgment

The authors are indebted to Richard Knowles, Taysir Jaouni, and Robert Patterson for assistance in the processing laboratory and to Marion Long, Henry Wright, and Earl Potter for nitrogen, solids, and reducing sugars analyses.

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The Design, Operation and Economic Evaluation of a Gas-Fired Lime Kiln

SYLVESTER M. HEINER[1]

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The common objective of all our company activities is to make a profit. In 1958 a survey of our operations at Nyssa and Nampa indicated that it would be profitable to increase our Steffens capacity at Nyssa. This was brought about by our increase in beets sliced at our Nyssa and Nampa factories, and the subsequent upset in molasses balance.

The increase in Steffens capacity called for:

One new cooler
Two additional saccharate drum filters 8′ diameter by 12′
Two new carb drum filters 8′ diameter by 14′
One new direct drum filter 8′ diameter by 14′
One new lime kiln of about 72 tons capacity of lime per day.

The large difference in BTU cost between gas and coke fuel indicated that a gas-fired kiln should be investigated.

Gas fuel available showed the following analysis: Nitrogen .7%, Carbon Dioxide .8%, Methane 91%, Ethane 5.9%, Propane 1.6%, Heat content 1042 BTU per cubic foot, Specific gravity .61.

Calculations show the optimum CO_2 for complete combustion of the above gas in air is 11.86%. The optimum combustion in air for straight carbon is 21%.

Research indicated that Victor J. Azbe[2] held patent numbers on the most desirable firing methods. Mr. Azbe offered his center-firing method, or multiple level side firing. We were reluctant to try center firing because it involved a tunnel beam through the center of the kiln. We were of the opinion that we might have clinkering difficulties above the center beam. We compromised on Mr. Azbe's multiple level side firing[3].

When using Mr. Azbe's BTU figure of 5 million BTU's per ton of lime, the maximum possible CO_2 is 32% by volume which requires an absorbtion of 72% at carbonation when using all available gas from the kiln. For our coke-fired kiln this figure is 62%. Previous carbonation absorbtion figures of 72% allowed

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[1] Chief Engineer, The Amalgamated Sugar Company, Ogden, Utah.

[2] Victor J. Azbe, 8135 Forsyth Blvd., St. Louis 24, Missouri.

[3] Victor J. Azbe holds patent numbers 2,199,384 dated May 7, 1940; 2,370,281 dated February 27, 1945; 2,532,077 dated November 28, 1950; and 2,742,276 dated April 17, 1956. Patents cover coal, oil and gas-firing; also multiple level firing.

us a margin for kiln draft control by paralleling both coke and gas kilns to carbonation.

A kiln cross section six feet wide by twelve feet long was selected. The width was limited to six feet due to the difficulty of obtaining gas penetration of the rock mass moving down the kiln. Gases have a tendency to rise vertically in the kiln. The 6×12 cross section of 72 square feet allows one square foot per ton of lime.

A rectangular steel shell was selected in place of an oval shell for the sake of simplicity. Figure 1 is a cross section of the kiln at the upper burner level. The use of the rectangular shell made it easier to sectionalize the brick work for easier repair which is standard practice in large soaking furnaces at the steel mills.

The inlet ports were sized to give a high inlet velocity. The ten lower ports were sized one and one-half inches square and twelve upper ports were sized two inches square. The 5/32" diameter gas jets were placed directly in front of the ports to force the gases into the kiln. This induces approximately one inch of additional draft across the ports. Normal gas pressure is five pounds on lower burners and eight to nine pounds on the upper burners. The normal flows are shown on Figure 3.

The next item was the selection of a suitable draw system. Three types were considered:

1. Vibrating chutes
2. A time volume system with a surge hopper to allow a sizeable draw at definite time intervals.
3. A continuous volume displacement system with a step control.

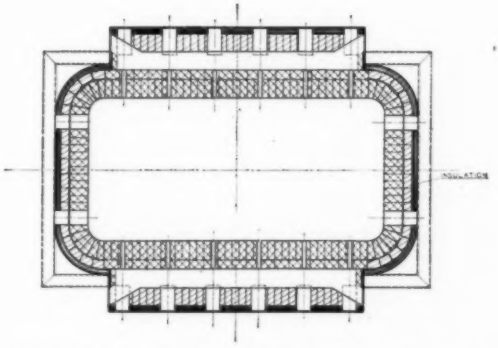

Figure 1.—Upper burner cross-section, rectangular gas-fired lime kiln.

Number 3, shown on Figure 2 was selected because this system makes a metered draw on each chute and approaches continuous operation.

The system consists of a hydraulic pump with an unloading device. The lime is displaced by means of plate feeders actuated by hydraulic cylinders. The system is controlled by a stepping switch which is operated by a return stroke limit switch. The

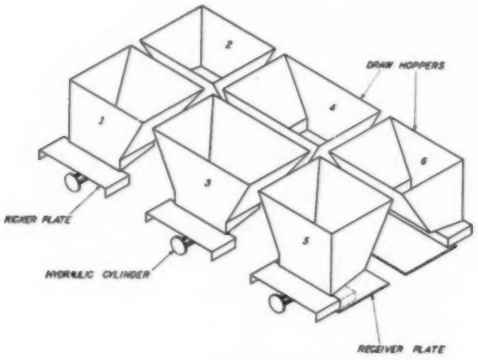

Figure 2.—Draw system.

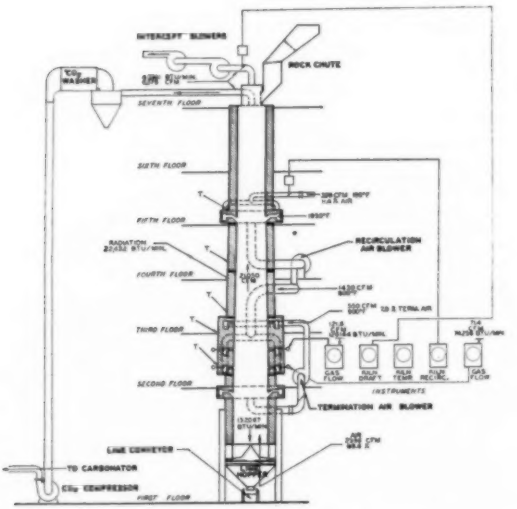

Figure 3.—Rectangular gas-fired lime kiln.

draw chutes are sequenced by the stepping switch and draw in numerical order. Each draw cylinder has to complete its 8″ stroke before the next chute starts. Draw rate is regulated by hydraulic flow control valves and the whole system is interlocked so that it stops or starts with the apron conveyor.

Previous research on the optimum burning time for Nyssa rock (size $3\frac{1}{2} \times 4\frac{1}{2}$) indicated an optimum burning time of 10 to 14 hours at 2,000 to 2,200° F. The calcining zone height was determined at 36 tons of CaO to allow 12 hrs. in the calcining zone.

A cross section of the lime kiln is shown in Figure 3. The lower ports are for air removal to the termination air fan. The second and third ports are the points at which the gas is introduced to the kiln along with the recirculation gases. Thirty-five to 40 percent of the gas is introduced at the lower burners and 60 to 65 percent is introduced at the upper burners. From 10 to 15 percent of the air required is introduced at the second and third port levels. At the fourth port level, heated air is admitted to terminate combustion. It is necessary to maintain uniform draw, draft and firing rate. If the draw rate is reduced, the fire zone will move up the kiln.

Instrumentation for the kiln consisted of two gas-flow recording meters; one for the twelve upper burners and one for the ten lower burners. The second instrument on the right (Figure 3) is a kiln draft recording controller which opens or closes the intercept damper to maintain the desired draft. The center instrument is a six point temperature recorder. By using a five point selector switch with each recording point, 30 temperatures can be measured in groups of six. The fourth instrument on the right is used to control and record the temperature of the re-circulation gases. This instrument also records the temperature of the exhaust gases from the kiln.

In order to check the level of rock in the kiln and thereby have a check on the draw system, we developed an automatic rock level recorder. This device consists of a long piece of shaft which is lowered into the kiln after each skip and automatically detects the rock level. The level is indicated on the first floor and recorded at a control panel.

At the present time the kiln is operated to produce 66 tons of lime per day.

Normal draft at kiln top 2.7″ to 3″ W.G.

Normal draft at recirculation outlet will be 2.5″ to 2.8″ W.G.

Normal Gases per Ton of Lime

Nitrogen	42,205 cubic feet @ 60° F.	30″ H.G.
Carbon Dioxide	17,830 cubic feet @ 60° F.	30″ H.G.
Oxygen	2,307 cubic feet @ 60° F.	30″ H.G.
Water Vapor	8,540 cubic feet @ 60° F.	30″ H.G.
Fuel to lower burners		37%
Fuel to upper burners		63%
Normal kiln outlet gas temperature		460 to 500° F.
Normal temperature at recirculation gas outlet		1650° F.
Normal recirculation temperature		800° F.

Kiln Heat Balance in BTU/min.

		BTU's per min.	*Percent*
Upper Burners		126,144	63%
Lower Burners		74,256	37%
Total Heat Input	(Item 1)	200,400	100%

Water Vapor Loss

480° F. and 60° F. reference 18.69 × 1130.4″		21,127	10.52%

Dry Products Loss

Nitrogen = 143.9 × .25 × (480-60 =		15,109	7.54%
Carbon Dioxide = 95.7 × .22 × (480-60) =		8,843	4.41%
Oxygen = 8.9 × .22 × (480-60) =		822	.41%
Total vapor and dry gas loss	(Item 2)	45,901	22.88%

Heating and Calcining

175 lbs. $CaCO_3$ heated from 40° F. to 1652° F. = 175 × .256 × 1612 =	72,218
Heat of dissociation at 1652° F. = 91.7 × 1249[4] =	114,533
Post heating lime to 2200° F. = 91.7 × .236 × (2200-1652) =	11,859
Total	198,610
Minus heat recovered from CO_2 (2200 to 480) = 75.47% CO_2 from rock; 72 lbs. × .25 × (1652-480) =	21,096

[4] 1249 from Lime and Limestone, Part I, by N. V. S. Knibbs and B. J. Gee.

Difference	(Item 3)	177,514	
Minus heat removed from 2200 ° F. of lime in cooler = 91.7 × .236 × 2100 =		45,447	
Useful output	(Item 4)	132,067	66%
Radiation loss by difference: Item 5 = Item 1 minus 2 + 4 =		22,432	11.12%
Kiln efficiency = $\frac{132067}{200400} \times 100$ =			66%
Kiln efficiency vapor removed = $\frac{132067}{179273} \times 100$ =			73.8%

This efficiency compares very favorably with gas kilns operating in the United States.

Another figure used for comparison between kilns is BTUs per ton of lime. For the Nyssa gas-fired vertical kiln this figure is 4.37 million BTUs per ton of lime, while gas-fired vertical kilns at Quincy, Illinois, run 4.8 million BTUs per ton of lime. A large company at Saint Genevieve, Missouri, operates fourteen gas-fired vertical lime kilns with a BTU figure of 5.8 to 6.0 million BTU per ton of lime. Gas-fired vertical lime kilns designed by Mr. Azbe at Branchton, Pennsylvania, achieve a figure of 4.2 million BTUs per ton of lime.

The highest efficiency figure for coke-fired kilns is 81% for a vertical kiln in Germany. This compares with 68.4% for our Nyssa coke-fired kiln and 73.5% for our Nampa coke-fired kiln.

The additional labor required of the gas-fired kiln over a comparable coke-fired kiln amounts to one man on the day shift. The cost of the gas-fired lime kiln over a coke-fired lime kiln amounted to approximately $46,000. The saving of gas over coke follows:

	1959	1960
Coke cost per therm	11.72¢	12.23¢
Coke in million BTU per ton rock	2.1	2.17
Gas in million BTU per ton rock	2.43	2.29
Coke cost per ton rock	$2.46	$2.57
Gas cost per ton rock	$ .84	$ .79
Saving per ton rock	$1.62	$1.78
Tons rock through gas kiln	16,750	17,000 (est.)

The additional cost of the gas-fired lime kiln over the installation of a comparable coke-fired lime kiln has been realized in fuel saving. It would be possible to use gas entirely in place of coke with suitable kiln, compressor and carbonator design.

Acknowledgments

Figures 1, 2 and 3.

W. H. Bingham—Draw System and Control,

J. H. Grantham—Kiln Charging System and Piping Detail,

G. S. Benford—Air Lock,

Engineering Department, The Amalgamated Sugar Company. Research and Operating Departments—The Amalgamated Sugar Company.

Evaluation of Combining Ability in Self-Fertile Lines of Sugar Beets Using Male-Sterile Testers

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The discovery of cytoplasmic male sterility in sugar beets by Owen (4)[2] stimulated interest in developing improved varieties of sugar beets through hybridization on a commercial scale. Although male sterility in open-pollinated material can be used to produce top-cross varieties, it is desirable to develop and use inbred lines to obtain maximum benefit from hybrid combinations. Since *Beta vulgaris* L. is normally a highly self-sterile species, the development of inbred lines is difficult without using the self-fertile gene reported by Owen (3) in 1942.

Using self-sterile material Stewart, Gaskill and Coons (8), and Doxtator and Skuderna (2) found that hybrid vigor can be expected with sugar beet crosses particularly in gross sugar per acre. In both instances the information on sucrose content was inconclusive but they agreed that possibly in some specific combinations increased sucrose percentage could be obtained. However, the general belief among sugar beet breeders has been that sucrose content is conditioned by additive factors with no expression of heterosis or dominance for sucrose content. Coons in 1936 (1) reported heterosis for yield in sugar beets and stated that in most instances among 41 F_1 hybrids the sucrose percentages approached the means of the parents. More recently, Powers, ET AL. (6) and Peterson and Dickenson (5) have reported the expression of heterosis for sucrose content among single crosses.

This study was undertaken to obtain additional information on the use of male-sterile lines to produce test crosses for evaluating combining ability of self-fertile inbred lines and to study hybrid vigor in regard to yield and sucrose content.

Materials and Methods

The test crosses to the male-sterile lines were made in 1956 using space isolations. One self-fertile pollen parent was planted in each of 18 isolations with the respective male-sterile tester parents. The tester parents were rogued prior to and during flowering to remove possible pollen producers. Seed was harvested individually from each tester and pollinator parent.

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[1] Assistant Plant Breeder and Plant Breeder, respectively, The Amalgamated Sugar Company, Nyssa, Oregon.

[2] Numbers in parentheses refer to literature cited.

Table 1.—Root Yields and Sucrose Percentages of Parental Lines.

Tester Parents	Yield Tons/Acre	Sucrose %
113H3	24.88	13.25
E322	23.55	14.22
E21	23.64	14.12
A2-90H0	20.22	13.92
175H15	18.00	14.90
Average	22.06	14.08
LSD .05	3.49	0.55
.01	4.70	0.74
Male Parents		
5-189	22.85	14.53
5-236	23.07	13.79
A2	20.30	13.97
5-235	19.05	13.92
5-185	17.23	14.20
5-207	15.15	14.19
5-181	14.33	13.17
5-197	13.76	14.31
5-180	13.64	13.78
5-231	13.05	13.90
5-238	13.03	13.91
5-239	12.30	13.32
5-237	11.56	13.88
5-204	11.15	12.54
5-205	10.28	13.92
5-208	10.28	13.72
5-188	9.96	13.84
5-194	9.54	13.97
Average	14.58	13.83
LSD .05	2.86	0.52
.01	3.86	0.70

The male-sterile testers used were as follows:

113H3[a]—A male-sterile line derived from US 22/3.

E322—An F_1 male sterile resulting from a cross between two self-sterile parents, 225H0[a] x C-51-1.

E21—An F_1 male sterile resulting from a cross between two self-fertile inbred lines, A2-90H0 x 19.2[a].

A2-90H0[a]—A male-sterile equivalent of a multigerm inbred line.

175H15[a]—A male-sterile equivalent of a monogerm inbred line.

The pollinator lines, with the exception of A2, were self-fertile inbred lines of diverse pedigrees. Fifteen of the lines were inbred three generations and two of them, 5-194 and 5-197, were inbred four generations. Lines 5-235 through 5-239 were closely

[a] These lines were developed by the USDA, ARS, Crops Research Division, Sugar Beet Section, Salt Lake City, Utah.

related, each descending from a different root selection from the same segregating F_2 material. A2 was an open-pollinated variety.

The experimental design for the progeny test was a randomized block with 12 replications. The parents were randomized within male and female groups and kept together as sub-blocks within each replication. Because of limited seed supplies of some lines and crosses, the plots were limited to single rows 40 ft. long. The field test was grown in 1957 at Nampa, Idaho.

Results

Parents:

A wide range in vigor was found among the pollen parents as shown by their respective yields in Table 1. Although they were planted in blocks apart from the more vigorous test crosses, biases were probably introduced because of competition between the single row plots used in the test. The range among the tester lines was not as great.

Table 2.—Gross Sugar per Acre for Test Crosses Between 18 Pollinator Lines and 5 Cytoplasmic Male-Sterile Testers. Figures Given Are Pounds per Acre.

Pollen Parents	Male-Sterile Testers					
	113H3	E322	E21	A2-90H0	175H15	Avg.
5-231	7840	8030	7300	7510	7500	7640[1]
5-181	7900	7230	7980	7990	7010	7630[1]
5-239	7910	7650	7620	8250	6350	7560
5-236	7500	7350	7720	7650	6720	7390
5-189	7600	7820	7910	7870	5570	7360
5-197	6800	7720	7990	8210	5960	7320
A2	7360	7370	7260	7060	7450	7300
5-207	6630	6840	7820	7350	7650	7260
5-194	6910	6720	7830	8350	6220	7220
5-235	7320	7610	7350	7370	6360	7210
5-180	7600	7240	7270	7220	6110	7090
5-185	7120	6540	7580	7240	6990	7090
5-238	6790	6640	7130	7560	6770	6990
5-204	7180	6740	7530	7110	6250	6970
5-188	7530	7020	7290	7350	5260	6910
5-208	7560	6610	6540	6920	6470	6820
5-205	6730	6710	7160	6730	6690	6810
5-237	6580	7190	7020	7240	5790	6780[1]
Avg.	7270	7180	7460[1]	7500[1]	6510[1]	7190
LSD .05	910	890	850	780	840	390
.01	N.S.	1200	N.S.	1060	1140	510

LSD between averages of testers .05 = 200, .01 = 270

Gross sugar per acre for check varieties: US22/3 = 6750, US35/2 = 6240

[1] Differ significantly from test average, .05.

Differences in sucrose content among the inbred lines were significant (.01) but the range was comparatively less than for yield. Omitting line 5-204 because the exceptionally low sucrose content is questionable, the two extremes differed by only 1.36 percent sucrose. The lack of a negative correlation between sucrose content and yield is noticeable among the self-fertile lines.

Test Crosses:

In evaluating the desirability of crosses, the greatest return per acre is the primary factor to consider. Gross sugar per acre is the best indirect measure of this but high gross sugar as a result of high sucrose content is preferred to high gross sugar due to high root yields.

The average of the five test crosses for each line was considered a measure of the general combining ability for the respective lines. Similarly, the measure of general combining ability for the testers was the average of the 18 crosses with the respective pollinator lines.

Table 3.—Sucrose Percentage of Test Crosses Between 18 Pollinator Lines and 5 Cytoplasmic Male-Sterile Testers.

Pollen Parents	Male-Sterile Testers					
	113H3	E322	E21	A2-90H0	175H15	Average
5-188	14.72	14.42	14.78	14.80	15.26	14.80[1]
5-231	14.89	14.22	14.65	14.75	15.21	14.74[1]
5-207	14.55	15.00	14.56	14.80	14.52	14.68
5-208	14.39	14.94	14.54	14.64	14.67	14.64
5-239	14.32	14.40	14.82	14.62	14.99	14.63
5-189	14.49	14.53	14.59	14.71	14.81	14.62
A2	14.45	14.52	14.62	14.59	14.76	14.59
5-197	14.35	14.61	14.72	14.62	14.43	14.55
5-204	13.88	14.55	14.46	14.36	14.93	14.44
5-238	14.31	14.28	14.31	14.21	14.93	14.41
5-205	13.93	14.28	14.00	14.09	15.55	14.37
5-236	13.92	14.46	14.44	14.41	14.63	14.36
5-237	13.62	14.44	14.17	14.33	15.09	14.33
5-194	13.90	14.15	14.41	14.22	14.98	14.33
5-235	13.91	14.12	14.62	13.99	14.80	14.29
5-181	14.59	14.02	14.20	14.00	14.52	14.27
5-180	14.13	14.48	14.28	14.22	14.25	14.27
5-185	14.02	14.14	14.34	13.96	14.18	14.13[1]
Average	14.24[1]	14.42	14.47	14.41	14.81[1]	14.47
LSD .05	0.68	N.S.	N.S.	0.57	0.62	0.28
.01	N.S.	N.S.	N.S.	0.75	0.81	0.37

LSD between averages of testers .05 = 0.15, .01 = 0.20

[1] Differ significantly from test average.

% Sucrose of check varieties: US22/3 = 13.78, US35/2 (824) = 14.51

Table 4.—Root Yields of Test Crosses Between 18 Pollinator Lines and 5 Cytoplasmic Male-Sterile Testers. Yields Are Given as Tons per Acre.

Pollen Parents	Male-Sterile Testers					
	113H3	E322	E21	A2-90H0	175H15	Average
5-181	27.1	25.8	28.1	28.5	24.1	26.73[1]
5-231	26.3	28.2	24.9	25.5	24.7	25.92
5-239	27.6	26.6	25.7	28.2	21.2	25.85
5-236	26.9	25.4	26.8	26.5	23.0	25.73
5-235	26.3	26.9	25.1	26.3	21.5	25.23
5-194	24.8	23.8	27.2	29.4	20.8	25.19
5-197	23.7	26.4	27.1	28.1	20.4	25.16
5-189	26.2	26.9	27.1	26.8	18.8	25.16
5-185	25.4	23.1	26.4	26.0	24.6	25.09
A2	25.5	25.4	24.8	24.2	25.2	25.02
5-180	26.9	25.0	25.5	25.4	21.4	24.85
5-207	22.8	22.8	26.8	24.8	26.3	24.71
5-238	23.7	23.2	24.9	26.6	22.7	24.24
5-204	25.8	23.2	26.0	24.8	20.9	24.14
5-205	24.2	23.5	25.6	23.9	21.5	23.71
5-237	24.2	24.9	24.8	25.3	19.2	23.66
5-188	25.6	24.4	24.7	24.8	17.2	23.33[1]
5-208	26.2	22.1	22.5	23.6	22.0	23.31[1]
Average	25.52	24.88	25.78[1]	26.04[1]	21.98[1]	24.83
LSD .05	2.9	2.8	2.7	2.5	2.6	1.25
.01	N.S.	3.8	N.S.	3.3	3.5	1.69

LSD between averages of testers .05 = 0.66, .01 = 0.89

Yields of check varieties: US22/3 = 24.5, US35/2 (824) = 21.5

[1] Differ significantly from test average, .05.

Tables 2, 3, and 4 give the results of the test crosses for gross sugar, percent sucrose, and root yields. Six lines were among the better general combiners both for gross sugar per acre and percent sucrose. These lines are 5-231, 5-239, 5-189, 5-197, 5-207, and A2.

Lines 5-181 and 5-236 were good combiners for yield and gross sugar per acre, but their hybrids were low in percent sucrose. Line 5-188 produced crosses that were high in sugar content but low in yield. These lines are not included in the following discussion.

The question arises as to the performance of the individual crosses among the six lines considered to have the best general combining ability. To study the effect of specific combining ability, the means of the individual crosses were adjusted by adding to or subtracting from them the deviation of the means of all respective crosses of each parent from the test averages. For example, the root yield of the cross 113H3 x 5-231 is adjusted as follows:

113H3 x 5-231 yielded 26.3 tons per acre
Test average = 24.83 T/A
All 5-231 crosses averaged 25.92 T/A
All 113H3 crosses averaged 25.52 T/A

Adjusted mean for 113H3 x 5-231 is as follows:

$$\overline{X}_a = 26.3 - (25.92 - 24.83) - (25.52 - 24.83)$$
$$= 24.5 \text{ tons per acre.}$$

The adjustment removes the general effect of both parents so that if specific effects did not occur the adjusted means of the individual crosses should equal the mean of the test . The deviations from the mean of the test then can be attributed to specific combining ability.

Table 5 gives the adjusted means of the test crosses for the six better general combining lines. It is noticeable with many of the crosses that a favorable specific reaction for yield is accompanied by an unfavorable reaction for sucrose percentage and vice versa. Crosses 113H3 x 5-231, E322 x 5-231. 175H15 x 5-207 and E322 x 5-207 are examples. Seven of the 30 crosses numeri-

Table 5.—Means of Crosses Involving the Better General Combining Lines Adjusted to Remove the General Effects of the Parents.

Pollen Parents	Male-Sterile Testers				
	113H3	E322	E21	A2-90H0	175H15
	Yield, Tons/A				
5-231	24.5	27.1[2]	22.9	23.2	*26.5*[2]
5-239	25.9[2]	25.6[2]	23.8	*26.0*[2]	23.1
5-189	*25.2*[1]	26.6[2]	25.9[2]	*25.3*[1]	21.4
5-197	22.7	*26.1*[2]	*25.9*[2]	*26.6*[2]	23.0
5-207	22.2	22.9	26.0[2]	23.7	29.3[2]
A2	24.6	25.2[1]	23.7	22.8	27.9[2]
Test Mean = 24.8					
	% Sucrose				
5-231	14.85[2]	14.00	14.38	14.54[1]	*14.60*
5-239	14.39	14.29	14.66[2]	*14.52*[1]	14.49[1]
5-189	*14.57*[1]	14.43	14.44	*14.62*[2]	14.32
5-197	14.50[1]	*14.58*[1]	*14.64*[2]	*14.60*[2]	14.01
5-207	14.57[1]	14.85[2]	14.35	14.65[2]	13.97
A2	14.56[1]	14.45	14.50[1]	14.53[1]	14.30
Test Mean = 14.47					

Italicized numbers are those that numerically exceed the test means for both yield and percent sucrose.

[1] Numerically above the test mean.

[2] Exceeds test mean at .05 level.

[3] Exceeds test mean at .01 level.

cally exceeded the test means for both yield and sucrose percentage (italicized in Table 5), with most differences being significant at .05 level of probability. In no instance did a cross which showed a large positive deviation from the test mean for yield also show a large positive deviation from the test mean for percent sucrose. The cross E21 x 5-197 gave deviations significant at .01 level of probability; however, its adjusted mean for yield was 3.3 tons per acre below the highest adjusted mean and 0.21 percent below the highest mean for percent sucrose.

Because there appeared to be a tendency for the higher yielding crosses to be down in sucrose, correlation coefficients were computed for yield vs. sucrose content using individual test cross means. Correlation coefficients were computed within testers and combined. The r values obtained were .11, —.36, —.13, —.22, —.39 and —.45 for crosses with 113H3, E322, E21, A2-90H0, 175H15 and combined, respectively. Only the combined value —.45 is significant (.05). This can be explained as being due to tester 175H15 whose crosses were high in sugar and low in yield in comparison to the others. However, within 175H15 the r value was not significant.

Although there is a negative trend in the yield-percent sucrose relationship it is not a strong association. Therefore, it should be possible to obtain high sugar-high yield combinations such as A2-90H0 x 5-197 and A2-90H0 x 5-239.

Relative Importance of General and Specific Combining Ability:

To gain further information on the relative importance of general and specific combining ability in this test, the sums of squares in the analysis of variance table (Table 6) for test crosses were divided into the female, male, and female x male components. The variance components were then computed as indicated in Table 6. The variance attributable to the males and females was considered an index of that part of the over-all variation among the test crosses due to the general combining ability of the parents. The interaction male x female variance was considered an index of that part of the over-all variation due to specific combining ability.

The variances obtained for the female parents s_F^2, show that the differences in general combining ability of the female parents were responsible for the greater part of the variation among the 90 test crosses, for both yield and sucrose content. Previously it was pointed out that 175H15 gave high sugar, low yielding crosses in comparison to the other male-sterile parents. Therefore, this parent probably contributed a large portion to the s_F^2 values.

Table 6.—Analysis of Variance Tables Showing a Breakdown of the Components of Mean Squares.

Source of Variation	D. F.	M. S.	M. S. Expectation	Variance Components
Yield				
Replications	11	708.9		
Test Crosses	89	1081.1		
Females	4	10,470.2	$S^2+12S_{FM}{}^2+216S_F{}^2$	$S_F{}^2 = 45.88$
Males	17	955.1	$S^2+12S_{FM}{}^2+ 60S_M{}^2$	$S_M{}^2 = 6.58$
Females × Males	68	560.5	$S^2+12S_{FM}{}^2$	$S_{FM}{}^2= 29.47$
Error	979	206.7	S^2	
Total	1079			
% Sucrose				
Replications	11	4.2397		
Test Crosses	89	1.4473		
Females	4	9.2332	$S^2+12S_{FM}{}^2+216S_F{}^2$	$S_F{}^2 = 0.039$
Males	17	2.1889	$S^2+12S_{FM}{}^2+ 60S_M{}^2$	$S_M{}^2 = 0.023$
Females × Males	68	0.8039	$S^2+12S_{FM}{}^2$	$S_{FM}{}^2= 0.018$
Error	979	0.5847	S^2	
Total	1079			

The general combining ability of the pollen parents caused comparatively less variation among the crosses in yield than in percent sucrose.

For yield the variance attributable to the interaction, $s_{FM}{}^2$, shows that specific combining ability was responsible for a greater portion of the variation than the male parents. This was not so in regard to sucrose. This would indicate that specific combining ability was of greater importance for yield than for sucrose content. For sugar content, general combining ability was of greater importance than specific combining ability of the parents.

Expression of Dominance and Heterosis:

The test crosses as a group are compared to the performance of the parents in Table 7. The averages of the test crosses for yield and percent sucrose were significantly above the averages

Table 7.—Average of 90 Test Crosses Compared to the Average Performance of the Male and Tester Parents.

	Yield, Tons/A	% Sucrose
Avg. of test crosses	24.83	14.47
Avg. of male parents	14.58	13.83
Avg. of tester parents	22.06	14.08

for either male or tester parents. In the absence of dominance or heterosis, the average of the test crosses should be approximately midway between the averages of the male and tester parents. Since this was not the case, it is assumed that dominance and/or heterosis exist for both yield and sucrose content.

To further study the parent-test cross relationship, individual test crosses were compared to the means of their respective parents, $\frac{P_1 + P_2}{2}$, and to the high parent. This was done for both yield and percent sucrose. If a test cross significantly exceeded the mean of its parents, it was considered to be an expression of dominance; but if the test cross was significantly above the high parent, it was considered to be an expression of heterosis. The remaining crosses were divided into two classes, those numerically above and those numerically below the mean of their respective parents. Table 8 shows the number of test crosses found in each category.

Table 8.—Classification of the 90 Test Crosses in Relation to the Performance of their Respective Parents.

	Yield of Roots	Percent Sucrose
Number showing heterosis	36	18
Number showing dominance	43	16
Of the remaining crosses:		
Number above $\frac{P_1 + P_2}{2}$	10	49
Number below $\frac{P_1 + P_2}{2}$	1	7

Most of the crosses showed either dominance of heterosis for yield of roots with only one cross yielding less than the mean of its parents. Furthermore, in checking the yields of the male parents (Table 1) with the averages of their respective test crosses (Table 4) little, if any, relationship was found between the vigor of the inbreds and the yield of their respective test crosses.

All but seven of the crosses were above the averages of their respective parents, $\frac{P_1 + P_2}{2}$, for percent sucrose. In addition, fewer crosses showed dominance or heterosis for sucrose content than for yield. Again, as with yield, there was little relationship between the sucrose content of the pollen parents (Table 1) and the sucrose content of their test crosses (Table 3).

These results emphasize the need for test crossing. In this test, parental performance would have provided a poor evaluation of the value of the inbreds for use in hybrid combinations.

Seven single crosses showed heterosis for both percent sucrose and yield. Of these, six were crosses to A2-90H0 and one to 175H15. However, only three of these crosses produced high gross sugar per acre. This means that heterosis PER SE does not insure a high yield of sugar per acre. In some cases, however, it may be an important factor in obtaining high gross sugar per acre from beets of high sucrose content.

Discussion:

The male-sterile testers used in this study were not consistent in evaluating the inbred lines for combining ability. The results indicate that a single tester line is inadequate for the determination of combining ability. However, if several testers are used the number of entries for a progeny test becomes unwieldy.

A possible solution would be to produce test crosses in a similar manner but to increase the quantity of single-cross seed produced. A preliminary test could then be made having a single entry representing each line to be tested. The one entry would consist of a blend of equal quantities of seed from all the test crosses for a particular line. After this preliminary screening, the remnant seed of the individual single crosses could be used for an additional progeny test to gain more precise information of the combining ability of the lines performing the best in the composite test.

It would also be desirable to use male-sterile testers that show promise for commercial use since specific combining ability may be relatively important particularly for yield. Thus desirable combinations found among the test crosses could be of immediate commercial value.

Based upon the results of Rounds, Rush ET AL. (7), percent sucrose should be given due emphasis when evaluating combining ability of sugar beet inbred lines since it is desirable to obtain high gross sugar per acre from beets that are high in sucrose content. The results of this test indicate that it is possible to isolate and identify such lines.

Specific combining ability for yield and the expression of heterosis or dominance for sucrose content may be important factors in obtaining hybrid combinations that will produce high gross sugar per acre with high sucrose content. Even so, knowing that they are factors to be considered, breeding programs can be arranged to place more emphasis on test crossing as a means of evaluating inbred lines and less on the performance of the lines themselves. The poor relationship between parental performance and the combining ability of the parental lines in this test also emphasizes the need for test crossing.

Summary

Seventeen self-fertile inbred lines and one open-pollinated variety of sugar beets were crossed to five cytoplasmic male-sterile tester lines. The crosses were planted in a field test with the parents included. The results of the test showed that there were differences among the inbred lines for general combining ability and that specific combining ability was important particularly in regard to yield. Heterosis and phenotypic dominance were found for both yield and sucrose content. Parental performance showed little association with combining ability indicating the necessity of making test crosses in evaluating inbreds.

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Notes Section

Coloring of black and white negatives or film positives for use in slide preparation. The differential coloring of various sections, columns, etc. of slide material intended for presentation to almost any audience can accomplish several things and at the same time present a much more pleasant object for the viewer. For example, a slide presenting data might have one or more figures the lecturer wishes to emphasize. These particular figures may be made to stand out from the balance in a column by simply using a different color on that particular one or few.

All this can be accomplished very simply using ordinary black and white methods of photography with a touch-up technique employing a common artist's brush and easily obtainable dyes, or in the case of film positives, a simple dipping method. The following method, written in cookbook fashion, is one developed in part and employed at the Agricultural Experiment Station of The Great Western Sugar Company at Longmont, Colorado.

Film Used: High Contrast Copy or Micro-File.

Exposure: Over expose to insure a very dense background. This insures that the dyes used later will not show where they go beyond the limits of the figures being colored.

Light distribution on any subject used to produce negatives for this purpose is extremely critical. "Hot spots" must be eliminated or a uniformly dense background will not be obtained.

Preparation and Coloring of Negatives: Rinse the negative in photo-flo solution for 5 minutes, allow to dry for several minutes, but not to the point of a completely dry emulsion. Mount the negative in any standard slide mount. This provides a handle and prevents fingerprints on the negative.

Food coloring such as is commonly used for dyeing of eggs, candies, cakes, cake frostings, etc. makes an entirely acceptable dye-stuff. They have an added advantage of being readily available and low in cost. Color is applied full strength with an "oo" water color brush to the figures, designs, etc. on the emulsion side of the film. Allow both film emulsion and color to dry thoroughly before use.

Background Coloring of Film Positives: The negative described above may be printed on film to produce a film positive. In this case, the background is clear and may be colored by complete immersion. Mix 8 drops of color in 6 ounces of water.

Immerse the film for 5 minutes or longer, dependent upon the intensity of background color desired. Dry the dyed film, insert in a slide mount and it is ready for use.

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